NiceZyme View of ENZYME: EC 3.2.1.4

Official Name

Cellulase.

Alternative Name(s)

Avicelase.

Beta-1,4-endoglucan hydrolase.

Beta-1,4-glucanase.

Carboxymethyl cellulase.

Celludextrinase.

Endo-1,4-beta-D-glucanase.

Endo-1,4-beta-glucanase.

Endoglucanase.

Reaction catalysed

Endohydrolysis of 1,4-beta-D-glucosidic linkages in cellulose, lichenin and cereal beta-D-glucans

Comment(s)

Will also hydrolyze 1,4-linkages in beta-D-glucans also containing 1,3-linkages.

Cross-references

Biochemical Pathways; map number(s)	A4
PROSITE	PDOC00511 ; PDOC00563 ; PDOC00565 ; PDOC00640 ; PDOC00877
BRENDA	3.2.1.4
PUMA2	3.2.1.4
PRIAM enzyme-specific profiles	3.2.1.4
Kyoto University LIGAND chemical database	3.2.1.4
IUBMB Enzyme Nomenclature	3.2.1.4
IntEnz	3.2.1.4
MEDLINE	Find literature relating to 3.2.1.4
MetaCyc	3.2.1.4

P40942, CEXY_CLOSR; P54583, GUN1_ACICE; P06566, GUN1_BACS4;
P07983, GUN1_BACSU; P20847, GUN1_BUTFI; P17877, GUN1_CLOJO;

UniProtKB/Swiss-Prot

Q04469, GUN1_CRYFL; Q12622, GUN1_HUMGT; P56680, GUN1_HUMIN;
P05522, GUN1_PERAE; P58599, GUN1_RALSO; P16216, GUN1_RUMAL;
P21833, GUN1_SCLSC; P33682, GUN1_STRHA; Q05156, GUN1_STRRE;
P13933, GUN1_STRSS; Q12714, GUN1_TRILO; P07981, GUN1_TRIRE;
P54424, GUN1_USTMA; P06565, GUN2_BACS4; P10475, GUN2_BACSU;
P37701, GUN2_CLOJO; P23666, GUN2_PERAE; P17974, GUN2_RALSO;
P21834, GUN2_SCLSC; P26222, GUN2_THEFU; P07982, GUN2_TRIRE;
P19570, GUN3_BACS4; P23549, GUN3_BACSU; P14250, GUN3_FIBSU;
Q12624, GUN3_HUMIN; P28622, GUN4_BACS5; Q07940, GUN4_RUMAL;
P26221, GUN4_THEFU; O14405, GUN4_TRIRE; O85465, GUN5_BACAG;
P43316, GUN5_HUMIN; Q01786, GUN5_THEFU; P43317, GUN5_TRIRE;
P22699, GUN6_DICDI; Q9C9H5, GUN9_ARATH; P37696, GUNA_ACEXY;
Q12679, GUNA_ASPKA; P22541, GUNA_BUTFI; P22534, GUNA_CALSA;
P07984, GUNA_CELFI; P17901, GUNA_CLOCE; P54937, GUNA_CLOLO;
P04955, GUNA_CLOTM; P23665, GUNA_FIBSU; P84194, GUNA_GLOTR;
P26414, GUNA_MICBI; P29719, GUNA_PAELA; P10476, GUNA_PSEFL;
P23660, GUNA_RUMAL; P27035, GUNA_STRLI; P19487, GUNA_XANCP;
P10474, GUNB_CALSA; P26225, GUNB_CELFI; P28621, GUNB_CLOCL;
P04956, GUNB_CLOTM; P46236, GUNB_FUSOX; P84196, GUNB_GLOTR;
Q12647, GUNB_NEOPA; P23550, GUNB_PAELA; P18126, GUNB_PSEFL;
P23661, GUNB_RUMAL; P14090, GUNC_CELFI; P37699, GUNC_CLOCE;
P23340, GUNC_CLOSF; P07985, GUNC_CLOTM; P46237, GUNC_FUSOX;
P27033, GUNC_PSEFL; P50400, GUND_CELFI; P25472, GUND_CLOCE;
P28623, GUND_CLOCL; P04954, GUND_CLOTM; P10477, GUNE_CLOTM;
Q05622, GUNE_RUMFL; P37698, GUNF_CLOCE; P26224, GUNF_CLOTM;
P46239, GUNF_FUSOX; P37700, GUNG_CLOCE; Q05332, GUNG_CLOTM;
P16218, GUNH_CLOTM; Q02934, GUNI_CLOTM; P45699, GUNK_FUSOX;
P55742, GUNM_CLOTM; Q59394, GUNN_ERWCA; P38686, GUNS_CLOTM;
P16630, GUNS_ERWCA; Q47096, GUNV_ERWCA; Q59395, GUNW_ERWCA;
P15329, GUNX_CLOTM; P38534, GUNX_PRUPE; P27032, GUNY_ERWCH;
P23659, GUNZ_CLOSR; P07103, GUNZ_ERWCH; P22669, GUN_ASPAC;
P06564, GUN_BACS1; P19424, GUN_BACS6; P29019, GUN_BACSZ;
P18336, GUN_CELUD; P15704, GUN_CLOSA; Q8X5L9, GUN_ECO57;
P37651, GUN_ECOLI; P82186, GUN_MYTED; P23548, GUN_PAEPO;
P22503, GUN_PHAVU; Q8RSY9, GUN_PSEFL; P23044, GUN_ROBSP;
Q8Z289, GUN_SALTI; Q8ZLB7, GUN_SALTY; P81190, GUN_SCHCO;
P58935, GUN_XANAC; P22533, MANB_CALSA; P84195, XYNA_GLOTR;

View entry in original ENZYME format

All UniProtKB/Swiss-Prot entries referenced in this entry, with possibility to download in different formats, align etc.
All ENZYME / UniProtKB/Swiss-Prot entries corresponding to 3.2.1.-
All ENZYME / UniProtKB/Swiss-Prot entries corresponding to 3.2.-
All ENZYME / UniProtKB/Swiss-Prot entries corresponding to 3.-

ExPASy Home page **Site Map** **Search ExPASy** **Contact us** **ENZYME** **Swiss-Prot**
Hosted by CBR Canada Mirror sites: Australia Brazil Korea Switzerland Taiwan USA

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Wednesday, February 08, 2006

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ	
☐	L9	insolen? same L5	0
☐	L7	hemicola same L5	0
☐	L5	microorganism same L4	20
☐	L4	(wash$5 or detergent or alkal$5 or laundr$5) same L3	202
☐	L3	(muta$5 or varia$5 or substitu$5 or delet$5) same L2	905
☐	L2	(gene or sequence or polynucleotide or recombinant) same L1	4608
☐	L1	(cellulase or (endoglucan same hydrolase)or glucanase or (carboxymethyl same cellulase) or endoglucanase)	15763

END OF SEARCH HISTORY

=> index bioscience medicine

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 11:13:20 ON 08 FEB 2006

=> s ((endoglucan(s)hydrolase#) or (carboxymethyl(s)cellulase#) or cellulase# or glucanase# or endoglucanase#)

4	FILE ADISCTI
1	FILE ADISNEWS
5240	FILE AGRICOLA
200	FILE ANABSTR
174	FILE ANTE
46	FILE AQUALINE
412	FILE AQUASCI
3793	FILE BIOENG
14277	FILE BIOSIS
7677	FILE BIOTECHABS
7677	FILE BIOTECHDS
4072	FILE BIOTECHNO
8413	FILE CABA
24389	FILE CAPLUS
2456	FILE CEABA-VTB
124	FILE CIN
376	FILE CONFSCI
206	FILE CROPB
433	FILE CROPU
98	FILE DDFB
60	FILE DDFU
9502	FILE DGENE
759	FILE DISSABS
98	FILE DRUGB
239	FILE DRUGMONOG2
84	FILE DRUGU
30	FILE EMBAL
4758	FILE EMBASE
3848	FILE ESBIOBASE
241*	FILE FEDRIP
81	FILE FOREGE
1306	FILE FROSTI
3484	FILE FSTA
5910	FILE GENBANK
25	FILE HEALSAFE
2158	FILE IFIPAT
81	FILE IMSPRODUCT
2263	FILE JICST-EPLUS
15	FILE KOSMET
5335	FILE LIFESCI
4885	FILE MEDLINE
14	FILE NIOSHTIC
385	FILE NTIS
144	FILE OCEAN
7445	FILE PASCAL
107	FILE PCTGEN
37	FILE PHIN
443	FILE PROMT
21	FILE RDISCLOSURE
11142	FILE SCISEARCH
1	FILE SYNTHLINE
2879	FILE TOXCENTER
9712	FILE USPATFULL
1035	FILE USPAT2
11	FILE VETB
411	FILE VETU
68	FILE WATER
3908	FILE WPIDS
17	FILE WPIFV

3908 FILE WPINDEX
18 FILE IPA
32 FILE NAPRALERT
190 FILE NLDB

L1 QUE ((ENDOGLUCAN(S) HYDROLASE#) OR (CARBOXYMETHYL(S) CELLULASE#) OR CELLUL
ASE# OR GLUCANASE# OR ENDOGLUCANASE#)

=> d rank
F1 24389 CAPLUS
F2 14277 BIOSIS
F3 11142 SCISEARCH
F4 9712 USPATFULL
F5 9502 DGENE
F6 8413 CABA
F7 7677 BIOTECHABS
F8 7677 BIOTECHDS
F9 7445 PASCAL
F10 5910 GENBANK
F11 5335 LIFESCI
F12 5240 AGRICOLA
F13 4885 MEDLINE
F14 4758 EMBASE
F15 4072 BIOTECHNO
F16 3908 WPIDS
F17 3908 WPINDEX
F18 3848 ESBIOBASE
F19 3793 BIOENG
F20 3484 FSTA
F21 2879 TOXCENTER
F22 2456 CEABA-VTB
F23 2263 JICST-EPLUS
F24 2158 IFIPAT
F25 1306 FROSTI

=> file f1-f4, f6-f9, f11-f14, f16, f19

FILE 'CAPLUS' ENTERED AT 11:17:37 ON 08 FEB 2006
COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'BIOSIS' ENTERED AT 11:17:37 ON 08 FEB 2006
Copyright (c) 2006 The Thomson Corporation

FILE 'SCISEARCH' ENTERED AT 11:17:37 ON 08 FEB 2006
Copyright (c) 2006 The Thomson Corporation

FILE 'USPATFULL' ENTERED AT 11:17:37 ON 08 FEB 2006
CA INDEXING COPYRIGHT (C) 2006 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'CABA' ENTERED AT 11:17:37 ON 08 FEB 2006
COPYRIGHT (C) 2006 CAB INTERNATIONAL (CABI)

FILE 'BIOTECHABS' ACCESS NOT AUTHORIZED

FILE 'BIOTECHDS' ENTERED AT 11:17:37 ON 08 FEB 2006
COPYRIGHT (C) 2006 THE THOMSON CORPORATION

FILE 'PASCAL' ENTERED AT 11:17:37 ON 08 FEB 2006
COPYRIGHT (C) 2006 INIST-CNRS. All rights reserved.

FILE 'LIFESCI' ENTERED AT 11:17:37 ON 08 FEB 2006
COPYRIGHT (C) 2006 Cambridge Scientific Abstracts (CSA)

FILE 'AGRICOLA' ENTERED AT 11:17:37 ON 08 FEB 2006

FILE 'MEDLINE' ENTERED AT 11:17:37 ON 08 FEB 2006

FILE 'EMBASE' ENTERED AT 11:17:37 ON 08 FEB 2006
Copyright (c) 2006 Elsevier B.V. All rights reserved.

FILE 'WPIDS' ENTERED AT 11:17:37 ON 08 FEB 2006
COPYRIGHT (C) 2006 THE THOMSON CORPORATION

FILE 'BIOENG' ENTERED AT 11:17:37 ON 08 FEB 2006
COPYRIGHT (C) 2006 Cambridge Scientific Abstracts (CSA)

=> s L1
L2 110974 L1

=> s (gene or sequence or polynucleotide or recombinant)(s)L2
7 FILES SEARCHED...
L3 17599 (GENE OR SEQUENCE OR POLYNUCLEOTIDE OR RECOMBINANT)(S) L2

=> s (muta? or varia? or modifi? or substitut? or delet?)(s)L3
7 FILES SEARCHED...
L4 4326 (MUTA? OR VARIA? OR MODIFI? OR SUBSTITUT? OR DELET?)(S) L3

=> s exprees? (s)L4
L5 0 EXPREES? (S) L4

=> s express? (s)L4
12 FILES SEARCHED...
L6 1632 EXPRESS? (S) L4

=> s (wash? or detergent? or alkal? or laundr?)(s)L6
L7 216 (WASH? OR DETERGENT? OR ALKAL? OR LAUNDR?)(S) L6

=> s microorganism# (s) L7
L8 45 MICROORGANISM# (S) L7

=> s hemicola (s) L8
L9 0 HEMICOLA (S) L8

=> dup rem l8
PROCESSING COMPLETED FOR L8
L10 41 DUP REM L8 (4 DUPLICATES REMOVED)

=> d ibib abs l10 1-41

L10 ANSWER 1 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 1
ACCESSION NUMBER: 2005-06332 BIOTECHDS
TITLE: New isolated recombinant glucanase polypeptide, useful in e.g., pulp treatment, food processing, animal feeds, preparing dough, preparing fuel products, and in brewing; recombinant enzyme protein production via plasmid expression in host cell for use in cellulose hydrolysis and nutritional supplement
AUTHOR: STEER B; CALLEN W; HEALEY S; PULLIAM D
PATENT ASSIGNEE: DIVERSA CORP
PATENT INFO: WO 2005003319 13 Jan 2005
APPLICATION INFO: WO 2004-US21492 2 Jul 2004
PRIORITY INFO: US 2003-484725 2 Jul 2003; US 2003-484725 2 Jul 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-112400 [12]
AN 2005-06332 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A ***glucanase*** polypeptide (I) has at least 50% ***sequence*** identity with any one of 259 polypeptide sequences (S1) over 20-100 residues, or is encoded by a nucleic acid having at least 50% ***sequence*** identity with any one of 259 ***polynucleotide*** sequences (S2) over 20-100 residues or by a nucleic acid hybridizing with any of (S2) under stringent conditions. The sequences are contained on a compact disk submitted with the application.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a nucleic acid (II) encoding (I) and having at least 50% ***sequence*** identity with respect to any one of (S2) over a region of 20-100 residues, or a ***sequence*** that hybridizes under stringent conditions to a nucleic acid comprising any one of (S2); (2) a nucleic

acid probe for identifying (II), comprising at least 10 consecutive bases of any one of (S2), or 10 consecutive residues of a nucleic acid ***sequence*** having at least 50% ***sequence*** identity to any one of (S2); (3) a primer pair for amplifying any of (II) or its subsequence; (4) a ***glucanase*** -encoding nucleic acid generated using the primer pair; (5) an isolated or ***recombinant*** ***glucanase*** encoded by the nucleic acid of (4); (6) an ***expression*** cassette comprising (II); (7) a vector comprising (II); (8) a cloning vehicle comprising (II); (9) a transformed cell comprising (II) or the ***expression*** cassette; (10) a transgenic non-human animal or transgenic plant (seed) comprising a ***sequence*** of (II); (11) an antisense oligonucleotide (VI) hybridizing under stringent conditions to a (sub) ***sequence*** of (II); (12) a double-stranded RNAi molecule comprising a subsequence of (II); (13) a polypeptide comprising (I) and lacking a signal ***sequence*** or a prepro ***sequence*** or having a heterologous signal or prepro ***sequence*** ; (14) a liquid, solid or gel protein preparation comprising (I); (15) a heterodimer or homodimer comprising (I); (16) an immobilized polypeptide comprising a (sub) ***sequence*** of (I); (17) an array comprising the immobilized polypeptide of (16); (18) an antibody that binds to (I); (19) a hybridoma producing the antibody; (20) producing polypeptide (I); (21) a computer system including a data storage device storing a ***sequence*** of (I) or (II); (22) a computer readable medium storing polypeptide or nucleic acid ***sequence*** of (I) or (II); (23) identifying a feature in a ***sequence*** or comparing sequences using a computer program operating on ***sequence*** data for (I) or (II); (24) generating a ***variant*** of (II); (25) modifying codons in (II) to increase or decrease its ***expression*** in a host cell; (26) producing a library of nucleic acids encoding multiple ***modified*** ***glucanase*** active sites or substrate binding sites; (27) producing a small molecule using (I) and other biosynthetic enzymes; (28) determining a functional fragment of ***glucanase*** enzyme by ***deleting*** amino acids from the ***sequence*** of (I) and testing for glycanase activity; (29) whole cell engineering of new or ***modified*** phenotypes using real-time metabolic flux analysis; (30) a signal ***sequence*** from any of (S1); (31) a chimeric polypeptide comprising the signal ***sequence*** of (30) and a peptide that is not naturally associated with the signal ***sequence*** ; (32) a nucleic acid encoding the chimeric polypeptide; (33) increasing thermotolerance or thermostability of ***glucanase*** polypeptide, involves glycosylating a ***glucanase*** , where the polypeptide comprises at least 30 continuous amino acids of (I); (34) overexpressing a ***recombinant*** ***glucanase*** in a cell using a high activity promoter, dicistronic vector or by ***gene*** amplification of the vector; (35) ***expressing*** a heterologous nucleic acid ***sequence*** in a plant cell, by transforming the cell with a heterologous nucleic acid ***sequence*** comprising ***sequence*** of (II), operably linked to a promoter, and growing the plant; (36) a dough or a bread product comprising (I); (37) a beverage comprising (I); (38) a food, a feed or a nutritional supplement comprising (I); (39) an edible enzyme delivery matrix comprising a thermostable ***recombinant*** ***glucanase*** enzyme; (40) delivering a ***glucanase*** supplement to an animal (41) a composition of cellulose or a cellulose derivative, comprising (I); (42) a wood, wood pulp or wood product, comprising (I); (43) a paper, paper pulp or paper product comprising (I); (44) a ***detergent*** composition comprising (I); (45) a pharmaceutical composition comprising (I); (46) a fuel comprising (I); (47) a dairy product comprising (I); and (48) an isolated or ***recombinant*** polypeptide comprising a ***sequence*** of (I), in which at least one amino acid residue is ***substituted*** .

BIOTECHNOLOGY - Preparation: (I) is produced by standard ***recombinant*** techniques in a bacterium, yeast, plant, insect, fungus or animal cell. The cell is preferably a Schizosaccharomyces pombe, Saccharomyces cerevisiae, Pichia pastoris, Escherichia coli, Streptomyces sp., Bacillus sp. or Lactobacillus sp. cell. Preferred Polypeptide: (I) has a ***glucanase*** activity, where the ***glucanase*** activity comprises an ***endoglucanase*** activity. The ***endoglucanase*** activity comprises an endo-1,4-beta- ***endoglucanase*** activity, or, involves catalyzing hydrolysis of

1,4-beta-D-glycosidic linkages or internal beta-1,3-glucosidic linkages. The 1,4-beta-D-glycosidic linkage activity involves hydrolysis of a 1,4-beta-D-glycosidic linkage in a cellulose, cellulose derivative, lichenin or a cereal. The cellulose derivative comprises a carboxyl methylcellulose or a hydroxy ethyl cellulose. The cereal comprises beta-D-glucan or a xyloglucan. The ***glucanase*** activity involves hydrolysis of polysaccharides comprising 1,4-beta-D-glycosidic-linked-D-glcopyranoses. The ***glucanase*** activity is thermostable or thermotolerant. (I) retains ***glucanase*** activity under conditions involving a temperature range of 1-5 degreesC, 5-15 degreesC, 15-25 degreesC, 25-37 degreesC, 37-95 degreesC, 55-85 degreesC, 70-95 degreesC, 70-75 degreesC or 90-95 degreesC. (I) retains a ***glucanase*** activity after exposure to a temperature of 1-5 degreesC, 5-15 degreesC, 15-25 degreesC, 25-37 degreesC, 37-95 degreesC, 55-85 degreesC, 70-75 degreesC or 90-95 degreesC or more. The ***glucanase*** activity comprises a specific activity at 37 degreesC in the range of 100-1000 units/mg of protein, 500-750 units/mg of protein, 500-1200 units/mg of protein, or 750-1000 units/mg of protein. The thermotolerance comprises retention of at least half of the specific activity of the ***glucanase*** at 37 degreesC after being heat to an elevated temperature. The thermotolerance comprises retention of specific activity at 37 degreesC in the range of 500-1200 units/mg of protein after being heat to an elevated temperature. (I) comprises one or more of glycosylation sites. The glycosylation is an N-linked glycosylation. (I) is glycosylated after being ***expressed*** inP. pastoris or S.pombe. (I) retains a ***glucanase*** activity under conditions of pH 6.5, 6.0, 5.5, 5.0, 4.5 or 4.0. (I) retains a ***glucanase*** activity under conditions comprising of pH 7.5, 8.0, 8.5, 9.0, 9.5, 10 or 10.5. Preferred Nucleic Acid: In (II), the ***sequence*** identity is at least 51-99% or more. The ***sequence*** identity is over a region of at least 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more residues, or the full length of a ***gene*** or a transcript. (II) comprises a nucleic acid ***sequence*** chosen from any one of (S2). The ***sequence*** comparison algorithm is a BLAST version 2.2.2 algorithm, where a filtering setting is set to blastall -p blastp -d nr pataa -F F, and all other options are set to default. (II) encodes (I) having ***glucanase*** activity, which is capable of catalyzing hydrolysis of 1,4-beta-D-glycosidic linkages or internal beta-1,3-glucosidic linkages, or, hydrolyzing a glucan to product a smaller molecular weight polysaccharide or oligomer. The ***glucanase*** activity comprises catalyzing hydrolysis of a glucan in a microbial cell, fungal cell, mammalian cell or plant cell. The nucleic acid having ***sequence*** that hybridizes under stringent conditions to a nucleic acid comprising any one of (S2), is at least 20, 30, 40, 50, 60, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more residues in length or the full length of the ***gene*** or transcript. The stringent conditions include a ***wash*** step comprising a ***wash*** in 0.2X saline sodium citrate at a temperature of 65degreesC for 15 minutes. In NA, the amplification is by polymerase chain reaction. The NA is generated by amplification of a ***gene*** library, where the ***gene*** library is an environmental library. Preferred Probe: The probe comprises an oligonucleotide having 10-50, 20-60, 30-70, 40-80, 60-100 or 50-150 consecutive bases. Preferred Primer: The primer comprises an oligonucleotide having at least 10-50 consecutive bases of any one of (S2), preferably 12-30 or more consecutive bases of any one of (S2). Preferred Cloning Vehicle: In the cloning vehicle the viral vector is an adenovirus vector, retroviral vector or an adeno-associated viral vector. The cloning vehicle is a bacterial artificial chromosome (BAC), plasmid, bacteriophage P1-derived vector (PAC), yeast artificial chromosome (YAC), or mammalian artificial chromosome (MAC). Preferred Transformed Cell: The cell is a bacterial, mammalian, fungal, yeast, insect or plant cell. Preferred Animal: The transgenic animal is a mouse. Preferred Plant: The plant is a corn plant, sorghum plant, potato plant, tomato plant, wheat plant, oilseed plant, rapeseed plant, soybean plant, rice plant, barley plant, grass or a tobacco plant. The seed is a corn seed, wheat kernel, oilseed, rapeseed, soybean seed, palm kernel, sunflower seed, sesame seed, rice, barley, peanut or a tobacco plant seed. Preferred RNA: RNAi comprises 15-25 or more duplex nucleotides in length. Preferred

Heterodimer: The second domain is a polypeptide and the protein is a fusion protein. The second domain is an epitope or a tag. Preferred Method: Determining differences between the first and second ***sequence*** further comprises the step of identifying polymorphisms. The method further comprises use of an identifier that identifies one or more features in a ***sequence*** , and involves reading the first ***sequence*** using a computer program and identifying one or more features in the ***sequence*** . Producing a ***variant*** of the polypeptide further involves ***expressing*** the ***variant*** of (II) to generate a ***variant*** ***glucanase*** polypeptide. The ***modification*** , additions or ***deletions*** are introduced by error-prone PCR, shuffling, oligonucleotide-directed ***mutagenesis*** , assembly PCR, sexual PCR ***mutagenesis*** , in vivo ***mutagenesis*** , cassette ***mutagenesis*** , recursive ensemble ***mutagenesis*** , exponential ensemble ***mutagenesis*** , site-specific ***mutagenesis*** , ***gene*** reassembly, ***Gene*** Site Saturation ***Mutagenesis*** , synthetic ligation reassembly (SLR) and their combination. The ***modification*** , additions or ***deletions*** are introduced by recombination, recursive ***sequence*** recombination, phosphothioate-***modified*** DNA ***mutagenesis*** , uracil-containing template ***mutagenesis*** , gapped duplex ***mutagenesis*** , point mismatch repair ***mutagenesis*** , repair-deficient host strain ***mutagenesis*** , chemical ***mutagenesis*** , radiogenic ***mutagenesis*** , ***deletion*** ***mutagenesis*** , restriction-selection ***mutagenesis*** , restriction-purification ***mutagenesis*** , artificial ***gene*** synthesis, ensemble ***mutagenesis*** , chimeric nucleic acid multimer creation and their combination. The method is iteratively repeated until a ***glucanase*** having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced. The ***variant*** ***glucanase*** polypeptide is thermotolerant, and retains some activity after being exposed to an elevated temperature. The ***variant*** ***glucanase*** polypeptide has increased glycosylation as compared to the ***glucanase*** encoded by a template nucleic acid. The ***variant*** ***glucanase*** polypeptide has a ***glucanase*** activity under a high temperature, where the ***glucanase*** encoded by the template nucleic acid is not active under the high temperature. The method is iteratively repeated until a ***glucanase*** coding ***sequence*** having an altered codon usage from that of the template nucleic acid, and higher or lower level of message ***expression*** or stability from that of the template nucleic acid is produced. Producing a small molecule comprises providing several biosynthetic enzymes capable of synthesizing or modifying a small molecule, where one of the enzymes comprises a ***glucanase*** enzyme encoded by (II), providing a substrate for at least one of the enzymes, and reacting the substrate with the enzymes under conditions that facilitate several biocatalytic reactions to generate a small molecule by series of biocatalytic reactions. Whole cell engineering of new or ***modified*** phenotypes using real-time metabolic flux analysis, comprises: (a) making a ***modified*** cell by modifying the genetic composition of a cell, where the genetic compositions is ***modified*** by addition of (II) to the cell; (b) culturing the ***modified*** cell to generate several ***modified*** cells; (c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and (d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions. (I) is useful for modifying a small molecule, which involves providing a ***glucanase*** enzyme comprising ***sequence*** of (I), providing a small molecule, reacting the enzyme with the small molecule under conditions that facilitate a enzymatic reaction catalyzed by the ***glucanase*** activity, thus modifying a small molecule by ***glucanase*** enzyme reaction. (I) or (II) is useful for producing an antibody to the protein, which involves administering (I) or (II) in an amount sufficient to generate a humoral immune response to non-human animal. Producing a transgenic plant comprises introducing a heterologous nucleic acid ***sequence*** comprising ***sequence*** of (II), into a cell, thus producing transformed plant cell, and producing a transgenic plant from the transformed cell. The method further involves

introducing the heterologous nucleic acid ***sequence*** by electroporation or microinjection of plant cell protoplasts, or introducing heterologous nucleic acid ***sequence*** directly to plant tissue by DNA particle bombardment or using an Agrobacterium tumefaciens host. The primers and probe are useful for identifying (II) in e.g., a water sample, liquid sample, soil sample, air sample, or a biological sample, where the biological sample is derived from a bacterial cell, protozoan cell, insect cell, yeast cell, plant cell, fungal cell or mammalian cell.

ACTIVITY - Antibacterial. No biological data given.

MECHANISM OF ACTION - None given.

USE - (I) is useful for hydrolyzing a cellulose (derivative) or a hemicellulose, e.g., in a wood, paper pulp or a paper product, and for catalyzing hydrolysis of glucan in a feed, food product or a beverage. The feed or feed product or beverage comprises a cereal-based animal feed, dough, wort or beer, or fruit or vegetable. (I) is useful for catalyzing hydrolysis of xylans in a microbial, plant cell, insect, yeast or mammalian cells. (I) is useful for catalyzing hydrolysis of a sugar to make a fuel product. (I) is useful for identifying its binding agents (e.g. modulators). (I) is useful as a nutritional supplement in animal diet, which involves preparing a nutritional supplement containing ***glucanase*** enzyme comprising at least thirty contiguous amino acids of (I), and administering the nutritional supplement to an animal to increase utilization of xylan contained in feed or food ingested by the animal, where the animal is a human, ruminant or a monogastric animal. (I) is useful for reducing lignin in a paper, wood or wood product. (I) is useful for eliminating or protecting animals from glucan containing ***microorganisms*** (especially Salmonella). (I) may also be used in oral care products. (I) is useful for producing or improving the flavor or texture of a dairy product. (I) is useful for producing small molecules (all claimed).

ADVANTAGE - (I) is thermostable or thermotolerant, and has increased ***glucanase*** activity at increased temperature and pH.

EXAMPLE - No relevant example is given. (289 pages)

L10 ANSWER 4 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-23482 BIOTECHDS
TITLE: New polypeptide having cellulolytic enhancing activity, useful for degrading or converting biomass to sugars and for producing organic substance and detergent composition; involving vector-mediated gene transfer and expression in host cell
AUTHOR: BROWN K; HARRIS P; ZARETSKY E; RE E; VLASENKO E; MCFARLAND K; LOPEZ DE LEON A
PATENT ASSIGNEE: NOVOZYMES INC
PATENT INFO: WO 2005074647 18 Aug 2005
APPLICATION INFO: WO 2005-US3525 28 Jan 2005
PRIORITY INFO: US 2004-540661 30 Jan 2004; US 2004-540661 30 Jan 2004
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-555779 [56]
AN 2005-23482 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide (I) having cellulolytic enhancing activity is new.

DETAILED DESCRIPTION - (I) comprises: (ILMV)-P-X(4,5)-G-X-Y-(ILMV)-X-R-X-(EQ)-X(4)-(HNQ) and (FW)-(TF)-K-(AIV) where X = any amino acid; X(4,5) = any amino acid at 4 or 5 contiguous positions; and X(4) = any amino acid at 4 contiguous positions where the polypeptide is selected from: (a) a polypeptide having an amino acid ***sequence*** which has at least 75% identity with amino acids 20-326 of a ***sequence*** of 326 amino acids (SEQ ID NO: 2), amino acids 18-240 of a ***sequence*** pf 478 amino acids (SEQ ID NO: 4), amino acids 20-258 of a ***sequence*** of 516 amino acids (SEQ ID NO: 6), amino acids 19-226 of a ***sequence*** of 452 amino acids (SEQ ID NO: 8), or amino acids 20-304 of a ***sequence*** of 608 amino acids (SEQ ID NO: 10), (b) a polypeptide which is encoded by a ***polynucleotide*** which hybridizes under at least medium stringency conditions with: (i) nucleotides 388-1332 of a ***sequence*** of 1846 bp (SEQ ID NO: 1), nucleotides 98-821 of a ***sequence*** of 880 bp (SEQ ID NO: 3),

nucleotides 126-978 of a ***sequence*** of 1000 bp (SEQ ID NO: 5), nucleotides 55-678 of a ***sequence*** of 681 bp (SEQ ID NO: 7), or nucleotides 58-912 of a ***sequence*** of 960 bp (SEQ ID NO: 9), (ii) the cDNA ***sequence*** contained in any of the sequences in (i), or (iii) a complementary strand of (i) or (ii), or (c) a ***variant*** comprising a conservative ***substitution*** , ***deletion*** , and/or insertion of one or more amino acid sequences in (a). INDEPENDENT CLAIMS are also included for: (1) producing a ***polynucleotide*** having a ***mutant*** nucleotide ***sequence*** ; (2) an isolated ***polynucleotide*** comprising a nucleotide ***sequence*** which encodes (I) or a ***mutant*** ***polynucleotide*** produced by the method of (1); (3) a nucleic acid construct comprising the ***polynucleotide*** operably linked to one or more control sequences that direct the production of the polypeptide in an ***expression*** host or a nucleic acid construct comprising a ***gene*** encoding a protein operably linked to a nucleotide ***sequence*** encoding a signal peptide consisting of nucleotides 330-387 of SEQ ID NO: 1, nucleotides 47-97 of SEQ ID NO: 3, nucleotides 69-125 of SEQ ID NO: 5, nucleotides 1-54 of SEQ ID NO: 7, or nucleotides 1-57 of SEQ ID NO: 9, where the ***gene*** is foreign to the nucleotide ***sequence*** ; (4) a ***recombinant*** ***expression*** vector comprising the nucleic acid construct of (3); (5) a ***recombinant*** host cell comprising the nucleic acid construct of (3); (6) producing (I); (7) producing a ***mutant*** of a parent cell; (8) a ***mutant*** cell produced by the method of (7); (9) producing a protein; (10) a transgenic plant, plant part or plant cell, which has been transformed with a ***polynucleotide*** encoding (I); (11) a ***detergent*** composition comprising the polypeptide having cellulolytic enhancing activity, a cellulolytic activity, and a surfactant;(12) degrading or converting a cellulosic material; and (13) producing an organic substance.

BIOTECHNOLOGY - Preferred Sequences: The isolated polypeptide further comprises: H-X(1,2)-G-P-X(3)-(YW)-(AILMV), (EQ)-X-Y-X(2)-C-X-(EHQN)-(FILV)-X-(ILV), or H-X(1,2)-G-P-X(3)-(YW)-(AILMV) and (EQ)-X-Y-X(2)-C-X-(EHQN)-(FILV)-X-(ILV), where X = any amino acid; X(1,2) = any amino acid at 1 position or 2 contiguous positions; X(3) = any amino acid at 3 contiguous positions; and X(2) = any amino acid at 2 contiguous positions. The polypeptide comprises an amino acid ***sequence*** which has at least 75-97% identity with amino acids 20-326 of SEQ ID NO: 2, amino acids 18-240 of SEQ ID NO: 4, amino acids 20-258 of SEQ ID NO: 6, amino acids 19-226 of SEQ ID NO: 8, or amino acids 20-304 of SEQ ID NO: 10. The polypeptide comprises any of the amino acid sequences of EVEN SEQ ID NOS: 2-10. The polypeptide consists of EVEN SEQ ID NOS: 2-10 or their fragments having cellulolytic enhancing activity. The polypeptide consists of amino acids 20-326 of SEQ ID NO: 2, amino acids 18-240 of SEQ ID NO: 4, amino acids 20-258 of SEQ ID NO: 6, amino acids 19-226 of SEQ ID NO: 8, or amino acids 20-304 of SEQ ID NO: 10. The polypeptide is encoded by a ***polynucleotide*** which hybridizes under at least medium-high or high stringency conditions with: (i) nucleotides 388-1332 of a ***sequence*** of 1846 bp (SEQ ID NO: 1), nucleotides 98-821 of a ***sequence*** of 880 bp (SEQ ID NO: 3), nucleotides 126-978 of a ***sequence*** of 1000 bp (SEQ ID NO: 5), nucleotides 55-678 of a ***sequence*** of 681 bp (SEQ ID NO: 7), or nucleotides 58-912 of a ***sequence*** of 960 bp (SEQ ID NO: 9), (ii) the cDNA ***sequence*** contained in any of the sequences in (i), or (iii) a complementary strand of (i) or (ii). The polypeptide is encoded by the ***polynucleotide*** contained in plasmid pEJG120 which is contained in E. coli NRRL B-30699, plasmid pTter61C which is contained in E. coli NRRL B-30813, plasmid pTter61D which is contained in E. coli NRRL B-30812, plasmid pTter61E which is contained in E. coli NRRL B-30814, or plasmid pTter61G which is contained in E. coli NRRL B-30811. The isolated ***polynucleotide*** has at least one ***mutation*** in the mature polypeptide coding ***sequence*** of ODD SEQ ID NOS: 1-9, in which the ***mutant*** nucleotide ***sequence*** encodes a polypeptide consisting of amino acids 20-326 of SEQ ID NO: 2, amino acids 18-240 of SEQ ID NO: 4, amino acids 20-258 of SEQ ID NO: 6, amino acids 19-226 of SEQ ID NO: 8, or amino acids 20-304 of SEQ ID NO: 10. The isolated ***polynucleotide*** is obtained by: (a) hybridizing a population of DNA under medium, medium-high, or high stringency conditions with: (i) nucleotides 388-1332 of SEQ ID NO: 1, nucleotides 98-821 of SEQ ID NO: 3,

nucleotides 126-978 of SEQ ID NO: 5, nucleotides 55-678 of SEQ ID NO: 7, or nucleotides 58-912 of SEQ ID NO: 9, (ii) the cDNA ***sequence*** contained in any of the nucleotides in (i), or(iii) a complementary strand of (i) or (ii), and (b) isolating the hybridizing ***polynucleotide*** , which encodes a polypeptide having cellulolytic enhancing activity. Preferred ***Mutant*** Cell: The ***mutant*** cell further comprises a ***gene*** encoding a native or heterologous protein. Preparation (claimed): Producing (I) comprises cultivating a cell, which in its wild-type form is capable of producing the polypeptide, or cultivating a host cell comprising a nucleic acid construct comprising a nucleotide ***sequence*** encoding the polypeptide, or a cell comprising the ***mutant*** ***polynucleotide*** of (2), under conditions conducive for production of the polypeptide, and recovering the polypeptide. Alternatively, the method comprises cultivating a transgenic plant or a plant cell comprising a ***polynucleotide*** encoding a polypeptide having cellulolytic enhancing activity under conditions conducive for production of the polypeptide, and recovering the polypeptide. Preferred Method: Producing a ***polynucleotide*** having a ***mutant*** nucleotide ***sequence*** comprises introducing at least one ***mutation*** into the mature polypeptide coding ***sequence*** of SEQ ID NO: 1, where the ***mutant*** nucleotide ***sequence*** encodes a polypeptide consisting of amino acids 20-326 of SEQ ID NO: 2, amino acids 18-240 of SEQ ID NO: 4, amino acids 20-258 of SEQ ID NO: 6, amino acids 19-226 of SEQ ID NO: 8, or amino acids 20-304 of SEQ ID NO: 10, and recovering the ***polynucleotide*** comprising the ***mutant*** nucleotide ***sequence*** . Producing a ***mutant*** of a parent cell comprises disrupting or ***deleting*** a nucleotide ***sequence*** encoding (I), which results in the ***mutant*** producing less of the polypeptide than the parent cell. Producing a protein comprises cultivating the ***mutant*** cell of (8) or the ***recombinant*** host cell of (5) under conditions conducive for production of the protein, and recovering the protein. Degrading or converting a cellulosic material comprises treating the cellulosic material with an amount of a cellulolytic protein in the presence of an amount of the polypeptide having cellulolytic enhancing activity, where the presence of the polypeptide having cellulolytic enhancing activity increases the degradation of cellulosic material compared to the absence of the polypeptide having cellulolytic enhancing activity. The cellulosic material is herbaceous material, agricultural residue, forestry residue, municipal solid waste, waste paper, or pulp and paper mill residue. The cellulosic material is corn stover. One or more cellulolytic enzymes are selected from ***cellulase*** , ***endoglucanase*** , cellobiohydrolase, or beta-glucosidase. The method further comprises treating the cellulosic material with an amount of one or more enzymes selected from hemicellulase, esterase, protease, laccase, peroxidase, or their mixture, where the esterase is a lipase, phospholipase, cutinase, or their mixture. The method is a pretreatment process. The method is a step in a simultaneous saccharification and fermentation process (SSF) or a step in a hybrid hydrolysis and fermentation process (HHF). The method further comprises recovering the degraded cellulosic material. The degraded cellulosic material is a sugar selected from glucose, xylose, mannose, galactose, or arabinose. The cellulolytic protein and/or the polypeptide having cellulolytic enhancing activity are in the form of a fermentation broth with or without cells. Producing an organic substance comprises: (a) saccharifying a cellulosic material with an amount of a cellulolytic protein in the presence of an amount of the polypeptide having cellulolytic enhancing activity, where the presence of the polypeptide having cellulolytic enhancing activity increases the degradation of cellulosic material compared to the absence of the polypeptide having cellulolytic enhancing activity, (b) fermenting the saccharified cellulosic material of step (a) with one or more fermentating ***microorganisms*** , and (c) recovering the organic substance from the fermentation. Steps (a) and (b) are performed simultaneously in a SSF. The organic substance is an alcohol, organic acid, ketone, amino acid, or gas, where the alcohol is arabinitol, butanol, ethanol, glycerol, methanol, 1,3-propanediol, sorbitol, orxylitol. The organic acid is acetic acid, acetonic acid, adipic acid, ascorbic acid, citric acid, 2,5 diketo-D-gluconic acid, formic acid, fumaric acid, glucaric acid, gluconic acid, glucuronic acid, glutaric

acid, 3-hydroxypropionic acid, itaconic acid, lactic acid, malic acid, malonic acid, oxalic acid, propionic acid, succinic acid, or xylonic acid. The ketone is acetone. The amino acid is aspartic acid, glutamic acid, glycine, lysine, serine, or threonine. The gas is methane, hydrogen, carbon dioxide, or carbon monoxide.

USE - The polypeptide, ***polynucleotide*** , and methods are useful for degrading or converting biomass to sugars, for producing an organic substance, and for producing ***detergent*** composition.

EXAMPLE - No relevant example given.(219 pages)

L10 ANSWER 6 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-01724 BIOTECHDS
TITLE: Novel purified cellulase e.g., mHKCel polypeptide, useful for treating wood pulp, for converting biomass to sugars, to generate high fructose corn syrup or for producing ethanol; vector-mediated cellulase gene transfer and expression in host cell for recombinant protein production and ethanol manufacture
AUTHOR: JONES B E; GRANT W D; HEAPHY S; GRANT S; REES H
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2004099370 18 Nov 2004
APPLICATION INFO: WO 2004-US13257 28 Apr 2004
PRIORITY INFO: US 2003-467315 30 Apr 2003; US 2003-467315 30 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-020802 [02]
AN 2005-01724 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A substantially purified mHKCel polypeptide (I) having biological activity of a ***cellulase*** , comprising a ***sequence*** chosen from an amino acid ***sequence*** having 85%, 90%, 95% or more ***sequence*** identity to a fully defined ***sequence*** (S1) of 571 amino acids as given in the specifications, (S1) and a substantially purified biologically active fragment of (S1), where the identity is determined by CLUSTAL-W program, is new.

DETAILED DESCRIPTION - A substantially purified mHKCel polypeptide (I) having biological activity of a ***cellulase*** , comprising a ***sequence*** chosen from an amino acid ***sequence*** having 85%, 90%, 95% or more ***sequence*** identity to a fully defined ***sequence*** (S1) of 571 amino acids as given in the specifications, (S1) and a substantially purified biologically active fragment of (S1), where the identity is determined by the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix. INDEPENDENT CLAIMS are also included for the following: (1) an isolated ***polynucleotide*** (II) chosen from (a) a nucleic acid ***sequence*** having 85% or more ***sequence*** identity to a fully defined ***sequence*** (S2) of 3796 nucleotides as given in the specification, or its complement, a nucleic acid ***sequence*** which encodes or is complementary to a ***sequence*** which encodes an mHKCel polypeptide having 85%, 90%, 95% or more ***sequence*** identity to (S1) and a nucleic acid ***sequence*** which encodes or is complementary to a ***sequence*** which encodes an mHKCel polypeptide having (S1), where (II) encodes (I), or (b) (S2) or its complementary ***sequence*** , a nucleic acid ***sequence*** that hybridizes, under high stringency conditions to (S2) or its complementary ***sequence*** or its fragment, a fully defined ***sequence*** (S3) of 1715 nucleotides as given in the specification or its complementary ***sequence*** , and a nucleic acid ***sequence*** that hybridizes, under high stringency conditions to (S3) or its complementary ***sequence*** or its fragment, where hybridization is conducted at 42degreesC in formamide (50%), SSC (6X), Denhardt's solution (5X), SDS (0.5%) and denatured carrier DNA (100 mug/ml) followed by ***washing*** two times in SSPE (2X) and SDS (0.5%) at room temperature and two additional times in SSPE (0.1) and SDS (0.5%) at 42degreesC; (2) an ***expression*** construct (III) comprising a ***polynucleotide*** ***sequence*** encoding an amino acid ***sequence*** having ***cellulase*** activity and having 85% or more ***sequence*** identity to (S1), or being capable of hybridizing to a probe designed to hybridize with (S3) under condition

of intermediate to high stringency, or being complementary to a nucleotide ***sequence*** having 85% or more sequences identity to a nucleotide ***sequence*** encoding (S1); (3) an ***expression*** vector (IV) comprising (II)-(a); (4) a vector comprising (III); (5) a host cell (V) transformed with (IV); (6) producing (I); (7) a ***recombinant*** host cell comprising a ***deletion*** or insertion or other alteration in mHKCel ***gene*** which is inactivates the ***gene*** and prevents mHKCel polypeptide production; (8) an antisense oligonucleotide (VI) complementary to a messenger RNA that encodes (I), where upon exposure to a ***cellulase*** -producing host cell, the oligonucleotide decreases or inhibits the production of ***cellulase*** by the host cell; (9) a ***detergent*** composition (VII) comprising (I) or surfactant and (I); (10) a feed additive comprising (I); (11) producing ethanol, involves contacting a biomass composition with an enzymatic composition comprising mHKCel to yield a sugar solution, adding to the sugar solution a fermentative ***microorganism*** , and culturing the fermentative ***microorganism*** under conditions sufficient to produce ethanol; and (12) identifying (I), involves isolating total microbial community DNA from an environment, constructing a genomic DNA library in Escherichia coli, screening the library for ***expression*** of ***cellulase*** activity, identifying the ***cellulase*** ***gene*** in the ***cellulase*** -positive clone, and characterizing (I).

BIOTECHNOLOGY - Preparation: (I) is prepared by culturing (V) such as bacterium (e.g., Streptomyces), filamentous fungi or yeast cell, in a suitable culture medium under suitable conditions to produce (I) and obtaining (I) (claimed). Preferred Polypeptide: (I) is obtained from Bacillus. Preferred ***Polynucleotide*** : (II)-(a) is chosen from mRNA, DNA, cDNA, genomic DNA, and its antisense analog. (II)-(a) is an RNA molecule. (II)-(a) encodes an enzyme having ***cellulase*** activity, where the enzyme is isolated from Trichoderma reesei. Preferred Vector: (IV) is operably linked to control sequences recognized by a host cell transformed with the vector. (IV) comprises a regulatory ***polynucleotide*** ***sequence*** including a promoter ***sequence*** derived from a glucose isomerase ***gene*** of Actinoplanes, a signal ***sequence*** derived from a Streptomyces ***cellulase*** ***gene*** , and (II). Preferred Oligonucleotide: In (VI), the host cell is a filamentous fungus.

USE - (I) is useful for treating wood pulp or for converting biomass to sugars, to generate high fructose corn syrup. (VII) is useful as ***detergent*** or dish ***detergent*** (claimed). (I) is useful for producing ethanol.

ADVANTAGE - (I) enables to improve the stability or activity of other enzymes involved in the degradation of plant cell wall material e.g., biomass, the value of animal feed or the drainability of wood pulp, enhance food product or reduce fiber in grain during the grain wet milling process or dry milling process.

EXAMPLE - To isolate DNA that encodes ***cellulase*** , samples of water were collected. The collected DNA was and was used for construction of the genomic DNA library. The purified DNA was partially digested with Sau3AI to give an average fragment size of 5 kb. The restricted DNA was size fractionated by electrophoresis on 0.5% agarose in TAE that comprised Tris-acetate (0.04 M) and EDTA (0.001 M). The restricted DNA was cloned into a Lambda vector using the ZAP-***Express*** vector kit. The primary libraries were amplified. The phagemid vector pBK-CMV was excised from the Lambda ZAP library using ExAssist helper phage and was used to infect Escherichia coli strain XLOLR. Plasmid-containing clones were isolated by plating on Luria - Bertani (LB) agar containing kanamycin (50 microg ml-1). DNA libraries in the pBK-CMV phagemid were screened for ***cellulase*** activity in a plate assay of the Escherichia coli clones. To detect ***cellulase*** activity the genomic libraries were plated on LB agar containing kanamycin. Positive clones exhibiting extracellular ***cellulase*** activity were surrounded by a yellow halo against a red background. The plasmid DNA was isolated from the positive clones, and the size of the inserts was determined by restriction digestion. DNA was sequenced using Applied Biosystems multisequence editor Seqed version 1.0.3. The result showed that DNA comprised a fully defined ***sequence*** of 3796 nucleotides as given in the specification, in which 1715 nucleotides pertained to ORF region, which encoded a fully defined ***sequence***

of 571 amino acids as given in the specification. (43 pages)

L10 ANSWER 7 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-01723 BIOTECHDS
TITLE: Novel substantially purified BagCel polypeptide, useful for treating wood pulp, for converting biomass to sugars, producing ethanol, identifying novel enzymes, as laundry detergent or dish detergent, for treating animal feed, paper; vector-mediated cellulase gene transfer and expression in host cell for recombinant protein production and ethanol manufacture
AUTHOR: JONES B E; GRANT W D; HEAPHY S; GRANT S
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2004099369 18 Nov 2004
APPLICATION INFO: WO 2004-US13175 28 Apr 2004
PRIORITY INFO: US 2003-467255 30 Apr 2003; US 2003-467255 30 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-020801 [02]
AN 2005-01723 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A substantially purified BagCel polypeptide (I) having ***cellulase*** activity, comprising a ***sequence*** having at least 85%, 90% or 95% ***sequence*** identity to a ***sequence*** (S1) of 570 amino acids fully defined in the specification, (S1), a substantially purified biologically active fragment of (S1), is new.

DETAILED DESCRIPTION - A substantially purified BagCel polypeptide (I) having ***cellulase*** activity, derived from Bacillus, comprising a ***sequence*** chosen from an amino acid ***sequence*** having at least 85%, 90% or 95% ***sequence*** identity to a ***sequence*** (S1) of 570 amino acids fully defined in the specification, (S1), a substantially purified biologically active fragment of (S1), where the identity is determined by the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix. INDEPENDENT CLAIMS are also included for: (1) an isolated ***polynucleotide*** (II) chosen from a nucleic acid ***sequence*** having at least 85% ***sequence*** identity to a ***sequence*** (S2) of 4205 nucleotides fully defined in the specification, or its complement, a nucleic acid encoding or complementary to a ***sequence*** encoding (I), with 85%, 90% or 95% ***sequence*** identity to (I); (2) an isolated ***polynucleotide*** (III) chosen from a nucleic acid ***sequence*** having (S2), or its complement, a nucleic acid ***sequence*** that hybridizes, under high stringency conditions to (S2), or its complement or fragment, a nucleic acid ***sequence*** having a ***sequence*** (S3) of 1713 nucleotides fully defined in the specification, or its complement, and a nucleic acid ***sequence*** that hybridizes, under high stringency conditions to (S3), or its complement or fragment, where (III) encodes a polypeptide having ***cellulase*** activity; (3) an ***expression*** construct (IV) comprising a ***polynucleotide*** ***sequence*** encoding an amino acid ***sequence*** having ***cellulase*** activity and having at least 85% ***sequence*** identity to (S1), or capable of hybridizing to a probe designed to hybridize with (S3) under conditions of intermediate to high stringency, or complementary to a nucleotide ***sequence*** having at least 85% ***sequence*** identity to a nucleotide ***sequence*** encoding (S3), where the identity is determined by the CLUSTAL-W program as above; (4) an ***expression*** vector (V) comprising (II), operably linked to control sequences recognized by a host cell transformed with the vector; (5) a vector (VI) comprising (IV); (6) a host cell (VII) transformed with (VI); (7) producing (M1) (I); (8) a purified enzyme (VIII) having ***cellulase*** activity produced by (M1); (9) a ***recombinant*** host cell (IX) comprising a ***deletion*** , insertion or other alteration in BagCel ***gene*** which inactivates the ***gene*** and prevents BagCel polypeptide production; (10) an antisense oligonucleotide (X) complementary to a mRNA that encodes an BagCel polypeptide having (S1), where upon exposure to ***cellulase*** -producing host cell, the oligonucleotide decreases or inhibits the production of ***cellulase*** by the host cell; (11) a

detergent composition (XI) comprising (I); (12) a ***detergent*** composition (XII) comprising surfactant and (I); and(13) a feed additive (XIII) comprising (I).

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (VII) in a suitable culture medium under suitable conditions to produce the ***cellulase*** , and obtaining the produced ***cellulase*** . (VII) is a filamentous fungi or yeast cell or bacterium. The bacterium is Streptomyces. Preferred ***Polynucleotide*** : (II) is chosen from mRNA, DNA, cDNA, genomic DNA and their antisense analog. (II) is an RNA molecule. (II) encodes an enzyme having ***cellulase*** activity, where the enzyme is isolated from Trichoderma source, preferably T.reesei. Preferred Vector: (V) comprises a regulatory ***polynucleotide*** ***sequence*** including a promoter ***sequence*** derived from glucose isomerase ***gene*** of Actinoplanes, a signal ***sequence*** derived from Streptomyces ***cellulase*** ***gene*** , and a ***polynucleotide*** ***sequence*** encoding a BagCel ***cellulase*** .

USE - (I) is useful for treating wood pulp, by contacting the wood pulp with (I). (I) is useful for converting biomass to sugars. The method further involves the generation of high fructose corn-syrup. (I) is useful for producing ethanol, by contacting the biomass composition with an enzymatic composition comprising (I) to yield a sugar solution, adding to the sugar solution a fermentative ***microorganism*** , and culturing the fermentative ***microorganism*** under conditions sufficient to produce ethanol. (I) is useful for identifying novel enzymes, by isolating total microbial community DNA from an environment, constructing a genomic DNA library in Escherichia coli, screening the library for ***expression*** of ***cellulase*** activity, and identifying the ***cellulase*** ***gene*** in the ***cellulase*** -positive clone, and characterizing the novel ***cellulase*** enzyme. (XI) is useful as ***laundry*** ***detergent*** or dish ***detergent*** (all claimed). (I) is useful in stone ***washing*** , modifying the texture, feel and/or appearance of cellulose-containing fabrics, for treating animal feed, pulp and/or paper, food and grain for grain wet milling process or dry milling process.

ADVANTAGE - (I) improves the smoothness or appearance of the fabric by removing pills and fibrils that tend to reduce the sharpness in appearance of a fabric. (I) is stable and improves the degradation of cell wall material such as cellulose or hemicellulose.

EXAMPLE - DNA libraries in the pBK-CMV phagemid were screened for ***cellulase*** activity in a plate assay in the Escherichia coli clones. To detect ***cellulase*** activity the genomic libraries were plated on LB agar containing kanamycin, carboxymethylcellulose (low viscosity sodium salt (0.5% w/v) and isopropyl-beta-D-thiogalactopyranoside (IPTG) (15 microl of a 0.5 M solution spread on the surface of the agar in a 7 cm diameter Petri dish). Following overnight growth at 37degreesC, the colonies were overlaid with 3ml molten agarose (0.7% w/v) dissolved in water cooled to 50degreesC. Then, the plates were flooded with Congo Red solution (0.1% w/v) for 30 minutes followed by 2 ***washes*** with 1 M NaCl. Positive clones exhibiting extracellular ***cellulase*** activity were surrounded by a yellow halo against a red background (R.Teather and P.J.Wood, Applied and Environmental Microbiology, 43:770-780, 1982). The screening of 110000 E.coli pBK-CMV clones yielded 4 zones of clearing indicating potential ***cellulase*** -producing colonies. Three ***cellulase*** -producing clones, after homogenizing the agar plug removed from the cleared zone, were streaked out for single colonies and confirmed the phenotype by the Congo Red test. Plasmid DNA was isolated from the three ***cellulase*** positive clones, and the size of the inserts determined by restriction digestion. All three clones had the same size (3.5 kb). The insert of environmental DNA of 4205 nucleotide bases was identified. The open reading frame (ORF) in the nucleotide ***sequence*** of the inserted environmental DNA of clone BagCel were identified using the ORF Find facility of the MapDraw program. The ORF had 1713 nucleotides corresponding to a protein of 570 amino acids. (42 pages)

L10 ANSWER 8 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-01023 BIOTECHDS
TITLE: Novel 029cel polypeptide with biological activity of

cellulase, useful as additive in detergent composition, in treatment of cellulose containing fabrics, in treatment of pulp and paper;
plasmid-mediated gene transfer and expression in fungus, yeast or bacterium for use in recombinant enzyme production for use in ethanol or corn-syrup production

AUTHOR: JONES B E; GRANT W D; HEAPHY S; GRANT S
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2004097001 11 Nov 2004
APPLICATION INFO: WO 2004-US13258 28 Apr 2004
PRIORITY INFO: US 2003-466831 29 Apr 2003; US 2003-466831 29 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-813903 [80]
AN 2005-01023 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A substantially purified 029cel polypeptide (I) with the biological activity of ***cellulase*** , comprising a ***sequence*** chosen from an amino acid ***sequence*** having at least 85 %, 90 % or 95 % ***sequence*** identity to or having a fully defined ***sequence*** of 581 amino acids (S1), as given in the specification, or a substantially purified biologically active fragment of (S1), where (I) or its derivative is obtained from Bacillus, is new.

DETAILED DESCRIPTION - A substantially purified 029cel polypeptide with the biological activity of ***cellulase*** , comprising a ***sequence*** chosen from an amino acid ***sequence*** having at least 85 %, 90 % or 95 % ***sequence*** identity to or having a fully defined ***sequence*** of 581 amino acids (S1), as given in the specification, or a substantially purified biologically active fragment of (S1), where (I) or its derivative is obtained from Bacillus and the identity is determined by CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix.

INDEPENDENT CLAIMS are also included for the following: (1) an isolated ***polynucleotide*** (II) chosen from: (a) a nucleic acid ***sequence*** having at least 85 % ***sequence*** identity to fully defined ***sequence*** of 3410 nucleotides (S2), as given in the specification, or its complement; (b) a nucleic acid ***sequence*** which encodes or is complementary to a ***sequence*** encoding (I); (c) a nucleic acid ***sequence*** having (S2), or its complement; (d) a nucleic acid ***sequence*** which hybridizes under high stringency conditions to (S2), or its complement or fragment; (e) a nucleic acid ***sequence*** having a fully defined ***sequence*** of 1746 nucleotides (S3), as given in the specification; and (f) a nucleic acid ***sequence*** that hybridizes under high stringency conditions to (S3), or its complement of fragment, where (II) encodes (I) and the hybridization is conducted at 42 degreesC in 50 % formamide, 6 X saline sodium citrate (SSC), 5 X Denhardt's solution, 0.5 % sodium dodecyl sulfate (SDS) and 100 microg/ml denatured carrier DNA followed by ***washing*** two times in 2 X SSPE (undefined) and 0.5 % SDS at room temperature and two additional times in 0.1 SSPE and 0.5 % SDS at 42 degrees C; (2) an ***expression*** construct (III), comprising a ***polynucleotide*** ***sequence*** encoding an amino acid ***sequence*** having ***cellulase*** activity and having at least 85 % ***sequence*** identity to (S1), or being capable of hybridizing to a probe designed to hybridize with (S3) under conditions or intermediate to high stringency, or being complementary to a nucleotide ***sequence*** having at least 85 % ***sequence*** identity to a nucleotide ***sequence*** encoding (S1); (3) an ***expression*** vector (IV) comprising (II) which is operably linked to control sequences recognized by a host cell transformed with the vector; (4) a vector (V) comprising (IV); (5) a host cell (VI) transformed with (IV); (6) producing (I); (7) a purified enzyme having ***cellulase*** activity prepared by the above method; (8) a ***recombinant*** host cell (VII) comprising a ***deletion*** or insertion or other alteration in the 029cel ***gene*** which inactivates the ***gene*** and prevents 029cel polypeptide production; (9) an antisense oligonucleotide (VIII) complementary to a messenger RNA that encodes an 029cel polypeptide (S1), where upon exposure to a ***cellulase*** -producing host cell, the oligonucleotide decreases or inhibits the production of ***cellulase***

by the host cell; (10) a ***detergent*** composition (IX) comprising (I) or (II) and a surfactant; (11) a ***detergent*** composition comprising a surfactant and a ***cellulase*** ;(12) a feed additive (X) comprising (I); (13) producing ethanol, by contacting a biomass composition with an enzymatic composition comprising 029cel to yield a sugar solution, adding to the sugar solution a fermentative ***microorganism*** , and culturing the fermentative ***microorganism*** under conditions sufficient to produce ethanol; and (14) identifying novel enzymes, by isolating total microbial community DNA from an environment, constructing a genomic DNA library in E.coli, screening the library for ***expression*** of ***cellulase*** activity, identifying the ***cellulase*** ***gene*** in the ***cellulase*** -positive clone, and characterizing the novel ***cellulase*** enzyme.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (VI) in a suitable culture medium under suitable conditions to produce the ***cellulase*** , and obtaining the produced ***cellulase*** . (IV) is a filamentous fungi or yeast cell or bacterium. The bacterium is Streptomyces. Preferred ***Polynucleotide*** : (II) is chosen from mRNA, DNA, cDNA, genomic DNA, and its antisense analog. (II) is an RNA molecule. (II) encodes an enzyme having ***cellulase*** activity, where the enzyme is isolated from a Trichoderma source. The enzyme is isolated from Trichoderma reesei. Preferred Vector: (IV) comprises a regulatory ***polynucleotide*** ***sequence*** including a promoter ***sequence*** derived from a glucose isomerase ***gene*** of Actinoplanes, a signal ***sequence*** derived from Streptomyces ***cellulase*** ***gene*** , and a ***polynucleotide*** ***sequence*** encoding a 029cel ***cellulase*** . Preferred Host Cell: (IV) is a prokaryotic or eukaryotic cell. Preferred Composition: (IX) is ***laundry*** ***detergent*** or dish ***detergent***

.

USE - (I) is useful for treating wood pulp which involves contacting the wood pulp with (I). (I) is useful for converting biomass to sugars which involves contacting the biomass with (I) and further comprises the generation of high fructose corn-syrup (claimed). (I) is useful as an additive in a ***detergent*** composition, in the treatment of cellulose containing fabrics, in the treatment of pulp and paper and in the treatment of starch for the production of high fructose corn-syrup or ethanol.

ADVANTAGE - (I) improves degradation of cell wall material e.g., cellulose and/or hemicellulose. (I) improves the stability or activity of other enzymes involved in degradation of plant cell wall material e.g., biomass.

EXAMPLE - The microbial flora were collected from water from Sonachi lake, Kenya. The DNA was extracted. Each DNA pellet was dissolved in 100 microl sterile Tris buffer 10 mM pH 8.5. The samples were pooled, giving a total of 20 microg DNA. The pooled DNA was used for construction of the genomic DNA library. The purified DNA was partially digested with Sau3A1 to give an average fragment size of 5 kbase. The restricted DNA was cloned into a lambda vector using the ZAP- ***Express*** (TM) vector kit and the Gigapak (TM) III Gold packaging extract. The primary libraries were amplified as per protocol by plating aliquots containing 5x104 plaque forming units (pfu) with host Escherichia coli strain XL1-Blue MRF' on 150mm Petri dishes and eluting the phage in buffer. Amplified libraries were stored in 7% v/v dimethyl sulfoxide at -80 degrees C after freezing in liquid nitrogen. The total primary titre was 1.8x106 pfu and after amplification 6.8x109 pfu/ml. The phagemid vector pBK-CMV was excised from the lambda ZAP library using ExAssist helper phage, and used to infect E.coli strain XLOLR. Plasmid-containing clones were isolated. DNA libraries in the pBK-CMV phagemid were screened for ***cellulase*** activity in a plate assay of the E. coli clones. To detect ***cellulase*** activity the genomic libraries were plated on Luria Broth (LB) agar containing kanamycin, 0.5 % w/v carboxymethylcellulose and isopropyl-beta-d-thiogalactopyranose (IPTG). Following overnight growth at 37 degrees C, the colonies were overlayed with 3 ml molten 0.7% w/v agarose dissolved in water which had been cooled to 50 degrees C. After this had set, the plates were flooded with 0.1 % w/v Congo Red solution for 30 minutes followed by 2 ***washes*** with 1 M NaCl. Positive clones exhibiting extracellular ***cellulase*** activity were surrounded by a yellow halo against a red background. The

screening of 110000 E. coli pBK-CMV clones yielded 4 zones of clearing indicating potential ***cellulase*** -producing colonies. Three of these were successfully recovered as ***cellulase*** -producing clones after homogenizing the agar plug removed from the cleared zone, streaking out for single colonies and confirming the phenotype by the Congo Red test. Plasmid DNA was isolated from the three ***cellulase*** positive clones, and the size of the inserts determined by restriction digestion. This identified an insert of environmental DNA of 3410 nucleotide bases. Possible open reading frames (ORF) in the nucleotide ***sequence*** of the inserted environmental DNA of clone 029cel were identified using ORF find facility of the MapDraw program. This identified an ORF composed of 1746 nucleotides corresponding to a protein of 581 amino acids, starting at position 3004 of the insert ***sequence*** and ending at position 1259. (41 pages)

L10 ANSWER 11 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-15198 BIOTECHDS
TITLE: New beta-glucosidase, for improving the characteristics of a yeast dough, producing ethanol, enhancing the cleaning ability of detergent compositions, improving the feel and appearance of cotton fabrics and as a softening agent; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production and food or fuel manufacture
AUTHOR: DUNN-COLEMAN N; WARD M
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2004043980 27 May 2004
APPLICATION INFO: WO 2003-US35672 5 Nov 2003
PRIORITY INFO: US 2002-424784 7 Nov 2002; US 2002-424784 7 Nov 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-411691 [38]
AN 2004-15198 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An isolated ***polynucleotide*** (I) derived from a fungal source, where the ***polynucleotide*** comprises a nucleotide ***sequence*** encoding an enzyme having beta-glucosidase (BGL6) activity, is new.
DETAILED DESCRIPTION - (I) comprises a nucleic acid ***sequence*** : (a) encoding BGL6 polypeptide having at least 85%, 90% or 95% ***sequence*** identity to a ***sequence*** comprising 838 amino acids (SEQ ID NO: 2); (b) comprising a ***sequence*** of 7468 bp (SEQ ID NO: 3) or its complement; or (c) that hybridizes, under high stringency conditions to the ***sequence*** of SEQ ID NO: 3, or to its complementary ***sequence*** , where (I) is derived from a Trichoderma source. INDEPENDENT CLAIMS are also included for: (1) an ***expression*** construct including (I); (2) a vector including the ***expression*** construct of (1) or comprising (I) operably linked to control sequences recognized by a host cell transformed with the vector; (3) a host cell transformed with the vector of (2); (4) a substantially purified BGL6 polypeptide with the biological activity of a beta-glucosidase comprising a ***sequence*** (i) having at least 85%, 90% or 95% ***sequence*** identity to the amino acid ***sequence*** of SEQ ID NO: 2, (ii) of SEQ ID NO: 2 or (iii) a substantially purified biologically active fragment of the amino acid ***sequence*** of SEQ ID NO: 2; (5) a method of producing an enzyme having beta-glucosidase activity;(6) a purified enzyme having beta-glucosidase activity prepared by the method of (5); (7) a ***recombinant*** host cell comprising (I) or a ***recombinant*** host cell comprising a ***deletion*** or insertion or other alteration in the bgl6 ***gene*** , which inactivates the ***gene*** and prevents BGL6 polypeptide production; (8) an antisense oligonucleotide complementary to a messenger RNA that encodes a BGL6 polypeptide, where upon exposure to a beta-glucosidase-producing host cell, the oligonucleotide decreases or inhibits the production of beta-glucosidase by the host cell; (9) a ***detergent*** composition comprising a polypeptide of (4); (10) a method of improving the characteristics of a yeast dough or baked good made from such dough; (11) a method of ***expressing*** a heterologous polypeptide having beta-glucosidase activity in an Aspergillus species; and (12) a method of producing ethanol.

BIOTECHNOLOGY - Preferred ***Polynucleotide*** : The % identity is calculated using the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix. Hybridization is conducted at 42degreesC in 50% formamide, 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 microg/ml denatured carrier DNA followed by ***washing*** two times in 2X SSPE and 0.5% SDS at room temperature and two additional times in 0.1 SSPE and 0.5% SDS at 42degreesC. The ***polynucleotide*** is an RNA molecule. The enzyme is derived from Trichoderma reesei. Preferred Host Cell: The host cell is a prokaryotic or a eukaryotic cell. The host cell is a filamentous fungi or yeast cell. Preferred Method: Producing an enzyme having beta-glucosidase activity comprises stably transforming a host cell with an ***expression*** vector comprising (I), cultivating the transformed host cell under condition for the host cell to produce the beta-glucosidase and recovering the beta-glucosidase. Improving the characteristics of a yeast dough or baked good made from such dough comprises mixing at least about 10 ppm of a BGL6 with dough ingredients to form a dough mixture and baking the dough mixture to form a baked good. Specifically, improving yeast bread dough or yeast roll dough or yeast bread or yeast roll characteristics comprises mixing at least about 10 ppm of a BGL6 with bread or roll dough ingredients to form a dough mixture, shaping or panning the dough mixture, proofing the dough mixture and baking the dough mixture to form bread or rolls. ***Expressing*** a heterologous polypeptide having beta-glucosidase activity in an Aspergillus species comprises providing a host Aspergillus with an ***expression*** vector comprising a ***polynucleotide*** encoding an Aspergillus beta-glucosidase signal ***sequence*** linked to a ***polynucleotide*** encoding a heterologous beta-glucosidase, thus encoding a chimeric polypeptide and cultivating the host Aspergillus under conditions for the Aspergillus to produce the chimeric polypeptide. Producing ethanol comprises: (a) contacting a biomass composition with an enzymatic composition comprising beta-glucosidase to yield a sugar solution; (b) adding to the sugar solution a fermentative ***microorganism*** ; and (c) culturing the fermentative ***microorganism*** under conditions to produce ethanol, where the biomass composition may be optionally pretreated. Step (a) further comprises the addition of at least one ***endoglucanase*** or cellbiohydrolase. The pretreatment is with a dilute acid.

USE - The ***polynucleotide*** and polypeptides and methods are useful in improving the characteristics of a yeast dough or baked good made from such dough, producing ethanol, enhancing the cleaning ability of ***detergent*** compositions, improving the feel and appearance of cotton fabrics and as softening agent.

EXAMPLE - A cDNA fragment, used as probe, was isolated by extracting total RNA from mycelia of a T. reesei grown under conditions to induce ***cellulase*** production and obtaining the polyadenylated (polyA) fraction. The polyA RNA was used to produce a cDNA pool, which was then, amplified using specific primers based on the bgl6 nucleic acid ***sequence*** . Northern blots were performed to confirm ***cellulase*** ***expression*** . The T. reesei RNA was used as template for RT-PCR. The mRNA was reverse transcribed to produce first strand cDNA. The cDNA served as template for PCR amplification of bgl6 cDNA ***sequence*** using specific oligonucleotide primers. The beta-glucosidase nucleic acid ***sequence*** comprises a ***sequence*** of 7468 bp (SEQ ID NO: 3) and the encoded protein comprising a ***sequence*** of 838 amino acids (SEQ ID NO: 2). (53 pages)

L10 ANSWER 12 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-16068 BIOTECHDS
TITLE: Novel BGL7 polypeptide having beta glucosidase activity useful for improving yeast dough or baked good made from such dough, for preparing detergent compositions or for enhancing potential aroma finished wine product;
recombinant fungus-derived beta-glucosidase useful for bacterium surface protein production
AUTHOR: DUNN-COLEMAN N; WARD M
PATENT ASSIGNEE: DUNN-COLEMAN N; WARD M
PATENT INFO: US 2004102619 27 May 2004

APPLICATION INFO: US 2002-301015 21 Nov 2002
PRIORITY INFO: US 2002-301015 21 Nov 2002; US 2002-301015 21 Nov 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-399738 [37]
AN 2004-16068 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A substantially purified BGL7 polypeptide (I) with the biological activity of beta-glucosidase, comprises the amino acid ***sequence*** chosen from an amino acid ***sequence*** having at least 85%, 90% or 95% ***sequence*** identity to a fully defined ***sequence*** (S1) of 765 amino acids as given in the specification, (S1) and a substantially purified biologically active fragment of (S1), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated ***polynucleotide*** (II) derived from a fungal source, comprising a nucleotide ***sequence*** encoding an enzyme having beta-glucosidase activity, (2) an isolated ***polynucleotide*** (III) chosen from a nucleic acid ***sequence*** which encodes or is complementary to a ***sequence*** which encodes (II) having at least 85%, 90% or 95% identity to (S1), a nucleic acid ***sequence*** which encodes or is complementary to a ***sequence*** which encodes (I) having (S1), a fully defined BGL7 nucleotide ***sequence*** (S2) as given in the specification, and a nucleic acid ***sequence*** that hybridizes under high stringency conditions to (S1), where the isolated ***polynucleotide*** encodes a polypeptide having the biological activity of a beta-glucosidase; (3) an isolated ***polynucleotide*** (IV) encoding an enzyme having beta-glucosidase activity, where the enzyme is derived from Trichoderma; (4) an ***expression*** construct including (V) including a ***polynucleotide*** ***sequence*** (I) having at least 85% ***sequence*** identity to (S1) or being capable of hybridizing to a probe derived from the nucleotide ***sequence*** having a fully defined ***sequence*** (S3) of 2377 nucleotides as given in the specification under conditions of intermediate to high stringency, or (III) being complementary to a nucleotide ***sequence*** having at least 85% ***sequence*** identity to (S1); (5) a vector (VI) comprising (V), or (III) operably linked to control sequences recognized by a host cell transformed with the vector; (6) a host cell (VII) transformed with (VI); (7) a ***recombinant*** host cell (VIII) comprising (III); (8) producing (I); (9) a ***recombinant*** host cell (IX) comprising a ***deletion*** or insertion or other alteration in the bg17 ***gene*** which inactivates the ***gene*** and prevents BGL17 polypeptide production; (10) an antisense oligonucleotide (X) complementary to a messenger RNA that encodes (I), where upon exposure to a beta-glucosidase-producing host cell, the oligonucleotide decreases or inhibits the production or beta-glucosidase by the host cell; (11) a ***detergent*** composition comprising (I); (12) ***expressing*** a heterologous polypeptide having beta-glucosidase activity in Aspergillus species, involves providing a host Aspergillus with an ***expression*** vector comprising a ***polynucleotide*** encoding Aspergillus beta-glucosidase signal ***sequence*** linked to a ***polynucleotide*** encoding a heterologous beta-glucosidase, thus encoding a chimeric polypeptide, cultivating the host Aspergillus under conditions suitable for Aspergillus to produce the chimeric polypeptide, where the chimeric polypeptide is produced; and (13) producing (M1) ethanol, involves: (a) contacting a biomass composition with an enzymatic composition comprising beta-glucosidase and a fermentative ***microorganism*** , and culturing the fermentative ***microorganism*** under conditions sufficient to produce ethanol, where the biomass composition may be optionally pretreated; or (b) contacting a biomass composition with an enzymatic composition comprising beta-glucosidase 4 to yield a sugar solution, adding to the sugar solution a fermentative ***microorganism*** , and culturing the fermentative ***microorganism*** under conditions sufficient to produce ethanol, where the biomass composition may be optionally pretreated.

BIOTECHNOLOGY - Preparation: (I) is prepared by stably transforming (VII) with (V) comprising (III), cultivating transformed host cell (VII) under conditions suitable for (VII) to produce (I), and recovering (I).

(VII) is a filamentous fungi or yeast cell (claimed). Preferred ***Polynucleotide*** : In (III), the percentage identity is calculated using the CLUSTAL-W program in MacVector version 6.5 operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix. The hybridization is conducted at 42degreesC in 50% formamide, 6xSSC, 5xDenhardt's solution, 0.5% SDS and 100 microg/ml denatured carrier DNA followed by ***washing*** two times in 2xSSPE and 0.5% SDS at room temperature and two additional times in 0.1 SSPE and 0.5% SDS at 42degreesC. (III) is an RNA molecule. In (IV), the enzyme is derived from T.reesei. Preferred Host Cell: (VII) is a prokaryotic cell or eukaryotic cell. Preferred Antisense Oligonucleotide: In (X), (VII) is a filamentous fungi. Preferred Method: (M1) further involves the addition of at least one cellbiohydrolase. The pretreatment is with a dilute acid.

USE - (I) is useful for improving the characteristics of a yeast dough or baked good made from such dough which involves mixing at least about 10 ppm of (I) with dough ingredients to form a dough mixture, and baking the dough mixture to form a baked good. (I) is useful for improving yeast bread dough or yeast roll dough or yeast bread or yeast roll characteristics which involves mixing at least about 10 ppm of (I) with bread or roll dough ingredients to form a dough mixture, shaping or panning the dough mixture, proofing the dough mixture, and baking the dough mixture to form bread or rolls (claimed). (I) is useful for preparing ***detergent*** compositions, for enhancing the potential aroma finished wine product, for hydrolysis of cellulose to its sugar components, for preparing ***cellulase*** composition for use in ***detergents*** or for degradation of biomass. (II), (III) or (IV) is useful for generating bacterial chimeric surface proteins, for identifying and characterizing related nucleic acid sequences or for production of disaccharides e.g., sophorose.

ADVANTAGE - (I) exhibits the same qualitative biological activity as the naturally occurring analog. (25 pages)

L10 ANSWER 14 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 2
ACCESSION NUMBER: 2004-02666 BIOTECHDS
TITLE: Manufacturing protein involves culturing recombinant microorganism comprising plasmid vector structural genes linked to promoter DNA fragments derived from alkali cellulase genes and isolating the protein;
recombinant protein production via plasmid expression in host cell
PATENT ASSIGNEE: KAO CORP
PATENT INFO: JP 2003265167 24 Sep 2003
APPLICATION INFO: JP 1993-107954 22 Jan 1993
PRIORITY INFO: JP 1993-107954 22 Jan 1993; JP 1993-25925 22 Jan 1993
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2003-857504 [80]
AN 2004-02666 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Manufacturing protein by culturing ***microorganism*** having a vector containing DNA encoding structural genes coupled downstream of DNA (I) promoting ***expression*** of a ***gene*** , where (I) is derived from promoter region of a fully defined Bacillus sp. ***alkali*** ***cellulase*** K-64 ***gene*** ***sequence*** of 639 nucleotides as given in specification or its ***mutant*** , and transit-peptide region, and isolating protein from culture.

DETAILED DESCRIPTION - Manufacturing protein by culturing ***microorganism*** having a vector containing DNA encoding structural ***gene*** coupled downstream of DNA (I) promoting ***expression*** of a ***gene*** , where (I) is derived from promoter region of a fully defined Bacillus sp. ***alkali*** ***cellulase*** K-64 ***gene*** ***sequence*** (S1) of 639 nucleotides as given in specification or a ***sequence*** of (S1) in which one or more nucleotides are ***deleted*** or ***substituted*** , and transit-peptide region, and isolating protein from culture.

WIDER DISCLOSURE - (1) a plasmid vector; and (2) a ***recombinant*** ***microorganism*** comprising the plasmid vector.

BIOTECHNOLOGY - Preferred Method: The structural ***gene*** involved in (M1) is acidic ***cellulase*** K330 ***gene*** , K-***alkali*** ***cellulase*** 635 ***gene*** or a chloramphenicol resistant ***gene*** .

USE - (M1) is useful for manufacturing protein by host ***microorganism*** such as Bacillus (Bacillus subtilis) (claimed).

ADVANTAGE - (M1) enables mass production of protein. isolated and were used for mass production of protein.

EXAMPLE - Mass production of ***alkali*** ***cellulase*** was performed as follows. ***Alkali*** ***cellulase*** ***gene*** fragment (HindIII ***gene*** fragment) was isolated from Escherichia coli, where the 4.4 kb fragment comprised ***alkali*** ***cellulase*** K-64 ***gene*** and promoter region. The fragment was contained in a plasmid pUHCL64. The HindIII fragment from the plasmid was introduced into a shuttle vector pHY300PLK to produce pHCL64. The ***recombinant*** plasmid (pHCL64a with HindIII fragment, or pHCL64b without the HindIII fragment) was introduced into Bacillus subtilis ISW1214 strain and was cultured in LB medium, and the ***alkali*** ***cellulase*** activity was measured by using the cell-free extract. The ***recombinant*** organism which comprised pHCL64a showed high ***expression*** of ***cellulase*** enzyme (13700 U/l) when compared with organism that comprised pHCL64b (3310 U/l). (15 pages)

L10 ANSWER 15 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 3
ACCESSION NUMBER: 2003-28637 BIOTECHDS
TITLE: New alkaline cellulase variants useful for producing a detergent composition used as a laundry detergent, an automatic dish washing detergent or a cotton fiber modifying detergent;
vector-mediated mutant enzyme gene transfer and expression in host cell for recombinant protein production and surfactant manufacture
AUTHOR: HAKAMADA Y; SAWADA K; ENDO K; KODAMA H; WADA Y; SHIKATA S; KOBAYASHI T
PATENT ASSIGNEE: KAO CORP
PATENT INFO: EP 1350843 8 Oct 2003
APPLICATION INFO: EP 2003-6842 27 Mar 2003
PRIORITY INFO: JP 2003-13840 22 Jan 2003; JP 2002-89531 27 Mar 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
AN 2003-28637 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An ***alkaline*** ***cellulase*** ***variant*** obtained by ***substituting*** the amino acid residue of a ***cellulase*** having an amino acid ***sequence*** exhibiting at least 90 % homology with a fully defined ***sequence*** of 824 amino acids (P1), as given in the specification, at position 10, 16, 22, 33, 39, 76, 109, 242, 263, 308, 462, 466, 468, 552, 564, or 608 in the ***sequence*** of P1, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) a ***gene*** encoding the ***alkaline*** ***cellulase*** ***variant*** ; (2) a ***recombinant*** vector comprising the ***gene*** ; (3) a transformant comprising the vector; (4) a ***detergent*** composition comprising the ***alkaline*** ***cellulase*** ***variant*** ; and (5) producing the ***alkaline*** ***cellulase*** ***variant*** ;

BIOTECHNOLOGY - Preparation (claimed): Producing the ***alkaline*** ***cellulase*** ***variant*** comprises ***expression*** of the ***gene*** encoding the ***alkaline*** ***cellulase*** ***variant*** under conditions that allow the ***expression*** of the encoded ***gene*** product, and recovery of the ***expressed*** ***alkaline*** protease. Preferred ***Cellulase*** ***Variant*** : The ***alkaline*** ***cellulase*** ***variant*** of a ***cellulase*** has an amino acid ***sequence*** exhibiting at least 90 % homology with the amino acid ***sequence*** of P1, and ***substitutions*** of the amino acid residues with another amino acid residue at position 10, 16, 22, 33, 39, 76, 109, 242, 263, 308, 462, 466, 468, 552, 564, or 608 in the ***sequence*** of P1, selected from the following amino acid residues:

(a) at position 10: glutamine, alanine, proline or methionine; (b) at position 16: asparagine or arginine; (c) at position 22: proline; (d) at position 33: histidine; (e) at position 39: alanine, threonine or tyrosine; (f) at position 76: histidine, methionine, valine, threonine or alanine; (g) at position 109: isoleucine, leucine, serine or valine; (h) at position 242: alanine, phenylalanine, valine, serine, aspartic acid, glutamic acid, leucine, isoleucine, tyrosine, threonine, methionine or glycine; (i) at position 263: isoleucine, leucine, proline or valine; (j) at position 308: alanine, serine, glycine or valine; (k) at position 462: threonine, leucine, phenylalanine or arginine; (l) at position 466: leucine, alanine or serine; (m) at position 468: alanine, aspartic acid, glycine or lysine; (n) at position 552: methionine; (o) at position 564: valine, threonine or leucine; or (p) at position 608: isoleucine or arginine. Preferred Transformant: A ***microorganism*** is used as a host.

USE - The ***alkaline*** ***cellulase*** ***variant*** is useful for the production of a ***detergent*** composition, such as a ***laundry*** ***detergent*** or a cotton fiber modifying ***detergent*** (claimed).

EXAMPLE - Random ***mutagenesis*** in a region of the ***alkaline*** ***cellulase*** ***gene*** was conducted by the error-prone PCR method, where the library of the ***variants*** was constructed. From the obtained ***variants*** , a ***variant*** effective for improving the production amount of the ***alkaline*** ***cellulase*** was selected. After determination of the ***mutation*** sites by nucleotide sequencing, a multiple ***variant*** was constructed by random ***mutagenesis*** or site-specific ***mutagenesis*** at the ***mutation*** sites by using appropriate primers. As the template DNA, an ***alkaline*** ***cellulase*** ***gene*** derived from the Bacillus sp. strain KSM-S237 introduced in a plasmid, pHY300PLK, was employed. The resulting PCR product was purified and the DNA mixture was collected. The DNA was introduced into the Bacillus subtilis strain ISW1214, where the corresponding transformant was obtained. The transformant having a halo formed on the DM3 regeneration agar plate was subjected to shake cultivation at 30 degrees centigrade for 15 hours in a polypeptone medium containing tetracycline. After collection of the cells, plasmids were collected and purified. The activity in the culture supernatant of each ***cellulase*** ***variant*** was assayed, and results revealed that an improvement in the production amount of most of the ***variants*** owes to an increase in the secretion amount of protein.

(28 pages)

L10 ANSWER 20 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-13365 BIOTECHDS
TITLE: Novel plasmids pBAL, pBAS2, pBAS isolated from extremely thermophilic, anaerobic Anaerocellum thermophilum, useful as shuttle vector and as expression vector for encoding heterogeneous protein such as amylase, cellulase; recombinant protein and recombinant enzyme production involving use of new shuttle vector plasmids
AUTHOR: AHRING B K; CLAUSEN A; MIKKELSEN M J
PATENT ASSIGNEE: UNIV DENMARK TECH DTU
PATENT INFO: WO 2003016536 27 Feb 2003
APPLICATION INFO: WO 2002-DK535 13 Aug 2002
PRIORITY INFO: US 2001-311871 13 Aug 2001; US 2001-311871 13 Aug 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-300618 [29]
AN 2003-13365 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Providing thermostable plasmid vectors as tools for genetic transformation of extremely thermophilic anaerobic ***microorganisms***

.

DETAILED DESCRIPTION - A plasmid (I) comprising any one of a fully defined pBAL, pBAS2 and pBAS nucleotide ***sequence*** of 8294 (S1), 3653 (S2) and 1863 (S3) nucleotides, respectively as given in the specification, or a nucleotide ***sequence*** which has more than 80% identity to (S1)-(S3), or nucleotide fragments comprising at least 20 nucleotides of (S1)-(S3), is new. INDEPENDENT CLAIMS are also included

for the following: (1) obtaining a shuttle vector pEAKS (II), involves digesting (I) with one or more restriction enzymes to obtain a fragment of the plasmid comprising a replication region, digesting a plasmid suited for the secondary host with one or more restriction enzymes to obtain a fragment of the plasmid comprising a replication region, and ligating the fragments to obtain a plasmid autonomously replicable in both the primary and secondary host; (2) a host cell (III) transformed with (I) or (II); (3) a protein (IV) encoded by any one of the open reading frames of (S1) or (S2), which comprises a fully defined ***sequence*** of 554, 192, 310, 135, 78, 67, 65, 61, 226, 60, 79, 80, 66, 330, 163, 109, 99 or 70 amino acids as given in the specification; and (4) a protein (V) which is a ***variant*** of the DNA polymerase/DNA repair protein having a fully defined ***sequence*** of 554 amino acids as given in the specification, where the amino acid identity between the protein and the DNA polymerase is at least 60% (more preferably 90%), the protein exhibiting DNA polymerase activity.

BIOTECHNOLOGY - Isolation: (I) is isolated from an extremely thermophilic ***microorganism*** or a thermophilic ***microorganism*** , such as Anaerocellum thermophilum (claimed). Preferred Plasmid: (I) comprises a marker ***gene*** such as beta-galactosidase ***gene*** or a drug resistance ***gene*** such as ampicillin resistance ***gene*** , kanamycin resistance ***gene*** , tetracycline resistance ***gene*** , erythromycin resistance ***gene*** , or chloramphenicol resistance ***gene*** . (I) comprises a multiple cloning site which comprises a restriction site for restriction enzymes EcoRI, HindIII, SacI, BamHI, XbaI, SalI or PstI. (I) comprises one or more ***expression*** control sequences. (I) also comprises a ***gene*** which has been inserted into it, and which codes for a heterogeneous protein, e.g. an enzyme such as alcohol dehydrogenase, carbohydrase, amylase, ***cellulase*** , beta-***glucanase*** , beta-glucosidase, alpha-glucosidase, xylanase, oxidoreductase, protease or a lipase. Preferred Host Cell: (III) is a thermophilic ***microorganism*** , preferably extremely thermophilic anaerobic ***microorganism*** . Preferably, the host is a bacterium chosen from Thermoanaerobacter, Thermoanaerobacterium, Thermoanaerobium, Thermoanaerobacteriodes, Anaerocellum, Caldicellusiruptor, Clostridium, Bacillus, Thermobacillus, Thermus and Thermotoga. Optionally, (III) is a mesophilic ***microorganism*** , such as Escherichia coli.

USE - (I) is useful as a shuttle vector comprising a replication region for use in a secondary host (e.g. a mesophilic ***microorganism*** , preferably Escherichia coli), different from the primary thermophilic host from which (I) having of (S1)-(S3) have been isolated. The shuttle vector has more than 60% (preferably 80%) nucleic acid identity to pEAKS. (III) is useful for producing a protein and recovering the protein, where the ***expressed*** protein confers the host cell with a changed phenotype, and in degradation or fermentation of biomaterial. (IV) or (V) is useful for the synthesis or repair of DNA (all claimed). (I) is useful as genetic ***expression*** system intended to operate at high temperatures. (I) is useful as ***expression*** vector for transforming host cells, and thus constructing ***microorganisms*** suitable for industrial production processes, at temperatures of 60-75degrees C for manufacturing ***recombinant*** proteins e.g. ***cellulases*** , amylases, xylanases, beta-galactosidases, beta-glucosidases, etc., which are subsequently recovered.

ADVANTAGE - Since the plasmids are derived from an extremely thermophilic anaerobic ***microorganism*** with temperature optimum of 72-75degrees C, they are extremely thermostable, and operate at high temperatures.

EXAMPLE - Screening for plasmids was done on thermophilic anaerobic isolates of DSM6725. Anaerobic cultures were grown in a BA medium as previously described Angelidaki et al., 1990, but the medium was amended with 1 g/l yeast extract and cysteine was not added. The medium was reduced with 0.25 g/l sodium sulfide. Appropriate carbon sources, cellulose, glucose, xylose, mannose or galactose was added at 5 g/l and incubation was at 70degrees C and pH 6.8. 60 ml overnight cultures were used for extraction of plasmids. Cells were harvested by centrifugation and ***washed*** once in 10 ml TE pH 8, pelleted and resuspended in TE containing 10 mg/ml lysozyme. The cells were lysed according to the method described by O'Sullivan et al., 1993 starting from step 2, and the

plasmid DNA was subsequently purified according to standard molecular methods. The plasmids pBAS2, pBAS and pBAL were detected and isolated from Anaerocellum thermophilum DSM6725. The presence of the plasmids, designated pBAS2, pBAS and pBAL, in A.thermophilum DMS6725 was verified by agarose gel electrophoresis using 1.2% agarose, stained with ethidium bromide and illuminated by UV light. For cloning purposes, the cloning vector pBluescript SK+ and cloning host Escherichia coli DH5-alpha was used. All three plasmids were characterized by digestion with 23 different restriction endonucleases. Plasmids pBAS and pBAL showed an unique EcoRI site and were cloned into EcoRI/SAP (shrimp ***alkaline*** phosphatase), treated pBluescript SK+ vector. pBAS was sequenced by plasmid walking. In the first round of sequencing the pBluescript KS+ primers T3 and T7 were used and the ***sequence*** information obtained was then used to design sequencing primers for the next round of sequencing pBAL revealed an additional unique SalI site, which was used for subcloning into SalI/EcoRI treated pBluescript KS+, eventually yielding two plasmids pBAAN 4.2 kb and pBAB 4 kb. These two plasmids were then sequenced by plasmid walking. Restriction mapping and sequencing of pBAS and pBAS2 revealed that pBAS was a plasmid sub-species of pBAS2. Since part of the nucleotide ***sequence*** of pBA2 was homologous to pBAS, the ***sequence*** of the unknown part of the plasmid was determined according to the following methods. The unknown part of pBAS2 was amplified using primers ccccctgcaggctattcttgtggggtagtgcag and ccccctgcagccttgaaacgcaaataaggtcc. This fragment was then sequenced by plasmid walking starting with primers ccttccacacgagcaggattcc and gctttttgattgcaagatggtattgc. The known part of the plasmid, common to pBAS, was verified by restriction enzyme digestion. The nucleotide residues 1-1863 of pBAS corresponded to nucleotide residues 638-2501 of pBAS2. The complete sequences of pBAL, pBAS2 and pBAS had 8294, 3653 and 1863 nucleotides, respectively as given in the specification. (64 pages)

L10 ANSWER 21 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-00137 BIOTECHDS
TITLE: New substantially purified BGL5 polypeptide with the biological activity of beta-glucosidase, useful for degrading biomass to ethanol;
recombinant enzyme protein production via plasmid expression in host cell useful for ethanol production
AUTHOR: DUNN-COLEMAN N; GOEDEGEBUUR F; WARD M; YAO J
PATENT ASSIGNEE: DUNN-COLEMAN N; GOEDEGEBUUR F; WARD M; YAO J
PATENT INFO: US 2003114330 19 Jun 2003
APPLICATION INFO: US 2001-26140 18 Dec 2001
PRIORITY INFO: US 2001-26140 18 Dec 2001; US 2001-26140 18 Dec 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-810909 [76]
AN 2004-00137 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A substantially purified BGL5 polypeptide (I) with the biological activity of a beta-glucosidase, comprising an amino acid ***sequence*** chosen from a fully defined ***sequence*** (S1) having 484 amino acids as given in specification, an amino acid ***sequence*** having at least 85%, 90% or 95% identity to (S1) and a substantially purified biologically active fragment of (S1), is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated ***polynucleotide*** derived from a fungal source, which comprises a nucleotide ***sequence*** encoding an enzyme having beta-glucosidase activity; (2) an isolated ***polynucleotide*** (II) chosen from a nucleic acid ***sequence*** which encodes or is complementary to a ***sequence*** which encodes (I), a Trichoderma reesei bgl5 nucleic acid coding ***sequence*** (S2) or its complement, and a nucleic acid ***sequence*** that hybridizes under stringent conditions to (S2) or its complement or fragment, where (II) encodes a polypeptide having the biological activity of beta-glucosidase; (3) an ***expression*** construct (III) including a ***polynucleotide*** ***sequence*** having at least 85% ***sequence*** identity to T.reesei bgl5 cDNA ***sequence*** (S3), or being capable of hybridizing to a probe derived from (S3) under conditions of intermediate to high stringency, or being complementary to a nucleotide ***sequence*** having at least 85% ***sequence***

identity to (S3); (4) a vector (IV) including (III); (5) a vector (V) comprising (II), operably linked to control sequences recognized by a host cell transformed with the vector; (6) a host cell (VI) transformed with (IV) or (V); (7) a ***recombinant*** host cell (VII) comprising (II); (8) producing (I); (9) a purified ***recombinant*** enzyme having beta-glucosidase activity; (10) a ***recombinant*** host cell comprising a ***deletion*** or insertion or other alteration in the bgl5 ***gene*** which inactivates the ***gene*** and prevents BGL5 polypeptide production; (11) an antisense oligonucleotide complementary to a messenger RNA that encodes a BGL5 polypeptide having (S1), where upon exposure to a beta-glucosidase-producing host cell, the oligonucleotide decreases or inhibits the production of beta-glucosidase by the host cell; (12) a ***detergent*** composition (VIII) comprising (I); and (13) ***expressing*** a heterologous polypeptide having beta-glucosidase activity in an Aspergillus sp., involves providing a host Aspergillus with an ***expression*** vector comprising a ***polynucleotide*** encoding a signal ***sequence*** linked to a ***polynucleotide*** encoding a heterologous beta-glucosidase, thus encoding a chimeric polypeptide, and cultivating the host Aspergillus under conditions suitable for the Aspergillus to produce the chimeric polypeptide, where the chimeric polypeptide is produced.

BIOTECHNOLOGY - Preparation: Producing (I), involves stably transforming a host cell with an ***expression*** vector comprising (II), cultivating the transformed host cell under condition suitable for the host cell to produce the beta-glucosidase, and recovering the beta-glucosidase. The host cell is filamentous fungi or yeast cell (claimed). Preferred ***Polynucleotide*** : In (II), the percentage identity is calculated using the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix. The ***polynucleotide*** is an RNA molecule. (II) encodes an enzyme having beta-glucosidase activity, where the enzyme is derived from a Trichoderma source (e.g., T.reesei). Preferred Host Cell: (VI) and (VII) are prokaryotic or eukaryotic cell.

USE - (I) is useful for producing ethanol, which involves contacting a biomass composition with an enzymatic composition comprising (I) to yield a sugar solution, adding to the sugar solution a fermentative ***microorganism*** , and culturing the fermentative ***microorganism*** under conditions sufficient to produce ethanol, where the biomass composition may be optionally pretreated. The method further involves the addition of at least one ***endoglucanase*** or cellbiohydrolase. The pretreatment is with a dilute acid. (I) is also useful for producing ethanol, which involves contacting a biomass composition with an enzymatic composition comprising (I) and a fermentative ***microorganism*** , and culturing the fermentative ***microorganism*** under conditions sufficient to produce ethanol, where the biomass composition may be optionally pretreated (claimed). (I) is useful in wine making for enhancing the potential aroma of the finished wine product. (VIII) is useful as softening agent, and also useful for improving the feel of cotton fabrics and for degrading wood pulp into sugars.

EXAMPLE - No relevant example is given. (21 pages)

L10 ANSWER 22 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08839 BIOTECHDS
TITLE: New purified EGVIII polypeptide with the biological activity of an endoglucanase, useful for producing ethanol and for degrading products made of cellulose;
recombinant enzyme protein production via plasmid expression in host cell for use in alcohol production
AUTHOR: DUNN-COLEMAN N; GOEDEGEBUUR F; WARD M; YAO J
PATENT ASSIGNEE: DUNN-COLEMAN N; GOEDEGEBUUR F; WARD M; YAO J
PATENT INFO: US 2003113735 19 Jun 2003
APPLICATION INFO: US 2001-28245 18 Dec 2001
PRIORITY INFO: US 2001-28245 18 Dec 2001; US 2001-28245 18 Dec 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-106462 [11]
AN 2004-08839 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A purified EGVIII polypeptide (I) with the biological activity of an ***endoglucanase*** comprising a fully defined ***sequence*** of 438 amino acids (S1) or 419 amino acids (S2) as given in the specification, an amino acid ***sequence*** which is 85%, 90%, or 95% identical to (S1), an amino acid ***sequence*** which is 95% identical to (S2), or a substantially purified biologically active fragment of (S2), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated egl8 ***polynucleotide*** (II) derived from a fungal source such as Trichoderma (III) and encoding (I) comprising a nucleic acid ***sequence*** encoding (I) or its complementary ***sequence*** , a nucleic acid ***sequence*** having a fully defined ***sequence*** of 1317 base pairs (S3) as given in the specification or its complementary ***sequence*** , or nucleic acid ***sequence*** hybridizing with (S3) or its complementary ***sequence*** or fragment under high stringent conditions; (2) ***expression*** vector (IV) containing a ***polynucleotide*** (V) which is at least 85% identical to (S1), ***polynucleotide*** which is capable of hybridizing to probe derived from nucleic acid ***sequence*** having a fully defined ***sequence*** of 1782 base pairs (S4) as given in the specification, under conditions of intermediate to high stringency or a ***polynucleotide*** which is complementary to (V); (3) vector (VI) containing (IV); (4) vector (VII) containing (II) operably linked to control ***sequence*** recognized by a host cell introduced with the vector; (5) host cell (VIII) transformed with (VI); (6) host cell (IX) transformed with (VII); (7) ***recombinant*** host cell (X) comprising (II); (8) ***recombinant*** enzyme having ***endoglucanase*** activity; (9) ***recombinant*** host cell comprising a ***deletion*** or insertion or other alteration in (II) ***gene*** which inactivates the ***gene*** and prevents (I) production; (10) an antisense oligonucleotide complementary to a mRNA that encodes (I) having (S2) wherein upon exposure to a ***endoglucanase*** -producing host cell, oligonucleotide decreases or inhibits the production of ***endoglucanase*** by host cell; (11) ***detergent*** composition comprising (I); (12) producing the enzyme having ***endoglucanase*** activity; and (13) ***expressing*** (M1) in heterologous polypeptide having ***endoglucanase*** activity in an Aspergillus sp. involves providing a host Aspergillus with an ***expression*** vector comprising ***polynucleotide*** encoding a signal ***sequence*** linked to a ***polynucleotide*** encoding a heterologous ***endoglucanase*** , thereby encoding a chimeric polypeptide and cultivating the host Aspergillus under suitable conditions to produce the chimeric polypeptide, thus the chimeric polypeptide is produced.

WIDER DISCLOSURE - Antibody specifically immunoreactive with (I) is also disclosed.

BIOTECHNOLOGY - Preparation: Producing an enzyme having ***endoglucanase*** activity involves stably transforming a host cell with an ***expression*** vector comprising a (II), cultivating transformed host cell under suitable conditions for host cell to produce ***endoglucanase*** and recovering the ***endoglucanase*** (claimed). Preferred ***Polynucleotide*** : Percentage identity of (II) is calculated using the CLUSTAL-W program in Mac vector version 6.5, operated with default parameters including an open gap penalty of 10.0 an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix.

USE - (I) is useful for producing ethanol which involves contacting a biomass composition with an enzymatic composition comprising ***endoglucanase*** preferably beta-glucosidase 4, addition of at least one ***endoglucanase*** preferably cellbiohydrolase to yield a sugar solution, and adding of fermentative ***microorganism*** to the obtained sugar solution or contacting biomass composition with an enzymatic composition comprising an ***endoglucanase*** preferably beta-glucosidase 4, a fermentative ***microorganism*** and addition of at least one ***endoglucanase*** preferably cellbiohydrolase, and culturing a fermentative ***microorganism*** under conditions sufficient to produce ethanol, where the biomass composition may be optionally pretreated with a dilute acid (claimed). (I) is useful in ***detergent*** compositions that exhibit enhanced cleaning ability,

function and softening agent and/or improve the feel of cotton fabrics. (I) is useful in compositions for degrading wood pulp into sugars and/or in feed compositions (I) is also useful for producing ethanol from agricultural waste, grasses and woods and other low-value biomass such as municipal waste and for degrading products made from cellulose in landfill.

ADVANTAGE - (I) efficiently degrades products made of cellulose.

EXAMPLE - cDNA fragment for use as a probe was isolated by extracting total RNA from mycelia of a Trichoderma reesei strain grown under conditions known to induce cellulose production and obtaining the polyadenylated fraction there from. The poly A RNA was used to produce a cDNA pool which was then amplified using specific primers based on the eg18 nucleic acid ***sequence*** . Total RNA was isolated form the mycelia using methods. Timberlake et al., 1981; Maniatis, et al., 1989; Ausubel, et al., 1993 and Sambrooke et al., 1989. Once isolated, Northern blots were performed to confirm cellulose ***expression*** and select an optimal induction time for cellulose ***expression*** and corresponding RNA isolation. messenger RNA (mRNA), having a poly (A) tail at the 3' end, may be purified from total RNA. The T.reesei RNA was used as template for RT-PCR using methods Loftus, J. et al., Science, 249:915-918, 1990. Thus the mRNA was reverse transcribed to produce first strand cDNA. The cDNA subsequently serves as template for PCR amplification of eg18 cDNA ***sequence*** using specific oligonucleotide primers designed in accordance with a fully defined ***sequence*** of 1782 base pairs or 1317 base pairs as given in the specification. (23 pages)

L10 ANSWER 23 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08838 BIOTECHDS
TITLE: New substantially purified EGVII polypeptide with the biological activity of a endoglucanase, useful for hydrolysis of cellulose to its sugar components;
recombinant enzyme protein production for use in ethanol production
AUTHOR: DUNN-COLEMAN N; GOEDEGEBUUR F; WARD M; YAO J
PATENT ASSIGNEE: DUNN-COLEMAN N; GOEDEGEBUUR F; WARD M; YAO J
PATENT INFO: US 2003113734 19 Jun 2003
APPLICATION INFO: US 2001-28244 18 Dec 2001
PRIORITY INFO: US 2001-28244 18 Dec 2001; US 2001-28244 18 Dec 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-106461 [11]
AN 2004-08838 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A substantially purified EGVII polypeptide (I) with the biological activity of ***endoglucanase*** , comprising a ***sequence*** chosen from a fully defined ***sequence*** of 249 amino acids (S1) as given in specification, an amino acid ***sequence*** having at least 85%, 90% or 95% ***sequence*** identity to (S1) or a substantially purified biologically active fragment of (S1), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated ***polynucleotide*** derived from a fungal source, comprising a nucleotide ***sequence*** encoding an enzyme having ***endoglucanase*** activity; (2) an isolated ***polynucleotide*** (II) chosen from a nucleic acid ***sequence*** which encodes or is complementary to a ***sequence*** which encodes (I), a Trichoderma reesei egl7 nucleic acid coding ***sequence*** (S2) or its complement, and a nucleic acid ***sequence*** that hybridizes under stringent conditions to (S2) or its complement or fragment, where (II) encodes a polypeptide having the biological activity of ***endoglucanase*** ; (3) an ***expression*** construct (III) including a ***polynucleotide*** ***sequence*** having at least 85% ***sequence*** identity to T.reesei egl7 cDNA ***sequence*** (S3), or being capable of hybridizing to a probe derived from (S3) under conditions of intermediate to high stringency, or being complementary to a nucleotide ***sequence*** having at least 85% ***sequence*** identity to (S3); (4) a vector (IV) including (III); (5) a vector (V) comprising (II), operably linked to control sequences recognized by a host cell transformed with the vector; (6) a host cell (VI) transformed

with (IV) or (V); (7) a ***recombinant*** host cell (VII) comprising (II); (8) producing (I); (9) a purified ***recombinant*** enzyme having ***endoglucanase*** activity; (10) a ***recombinant*** host cell comprising a ***deletion*** or insertion or other alteration in the egl7 ***gene*** which inactivates the ***gene*** and prevents EGVII polypeptide production; (11) an antisense oligonucleotide (VIII) complementary to a mRNA that encodes EGVII polypeptide having (S1), where upon exposure to a ***endoglucanase*** -producing host cell, the oligonucleotide decreases or inhibits the production of ***endoglucanase*** by the host cell; (12) a ***detergent*** composition (IX) comprising (I); and (13) ***expressing*** a heterologous polypeptide having ***endoglucanase*** activity in an Aspergillus sp., involves providing a host Aspergillus with an ***expression*** vector comprising a ***polynucleotide*** encoding a signal ***sequence*** linked to a ***polynucleotide*** encoding a heterologous ***endoglucanase*** , thus encoding a chimeric polypeptide, and cultivating the host Aspergillus under conditions suitable for the Aspergillus to produce the chimeric polypeptide, where the chimeric polypeptide is produced.

BIOTECHNOLOGY - Preparation: Producing (I), involves stably transforming a host cell with an ***expression*** vector comprising (II), cultivating the transformed host cell under condition suitable for the host cell to produce the ***endoglucanase*** , and recovering the ***endoglucanase*** . The host cell is filamentous fungi or yeast cell (claimed). Preferred ***Polynucleotide*** : In (II), the percentage identity is calculated using the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix. The ***polynucleotide*** is an RNA molecule. (II) encodes an enzyme having ***endoglucanase*** activity, where the enzyme is derived from a Trichoderma source (e.g., T.reesei). Preferred Host Cell: (VII) is prokaryotic or eukaryotic cell.

USE - (I) is useful for producing ethanol which involves contacting a biomass composition with an enzymatic composition comprising (I) to yield a sugar solution, adding to the sugar solution a fermentative ***microorganism*** , and culturing the fermentative ***microorganism*** under conditions sufficient to produce ethanol, where the biomass composition may be optionally pretreated. The method further involves the addition of at least one ***endoglucanase*** or cellbiohydrolase. The pretreatment is with a dilute acid. (I) is also useful for producing ethanol, which involves contacting a biomass composition with an enzymatic composition comprising (I) and a fermentative ***microorganism*** , and culturing the fermentative ***microorganism*** under conditions sufficient to produce ethanol, where the biomass composition may be optionally pretreated (all claimed). (I) is useful for hydrolysis of cellulose to its sugar components. (IX) is useful as softening agent, and also useful for improving the feel of cotton fabrics and for degrading wood pulp into sugars.

EXAMPLE - No relevant example is given. (22 pages)

L10 ANSWER 24 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08837 BIOTECHDS
TITLE: New substantially purified EGVI polypeptide with the biological activity of endoglucanase, useful for degrading biomass to ethanol;
recombinant enzyme protein production and fermentation for use in alcohol production
AUTHOR: DUNN-COLEMAN N; GOEDEGEBUUR F; WARD M; YAO J
PATENT ASSIGNEE: DUNN-COLEMAN N; GOEDEGEBUUR F; WARD M; YAO J
PATENT INFO: US 2003113732 19 Jun 2003
APPLICATION INFO: US 2001-26994 18 Dec 2001
PRIORITY INFO: US 2001-26994 18 Dec 2001; US 2001-26994 18 Dec 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-106460 [11]
AN 2004-08837 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A substantially purified EGVI polypeptide (I) with the biological activity of an ***endoglucanase*** , comprising an amino acid ***sequence*** chosen from a fully defined ***sequence***

(S1) having 837 amino acids as given in specification, an amino acid ***sequence*** having at least 85%, 90% or 95% identity to (S1) and a substantially purified biologically active fragment of (S1), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) An isolated ***polynucleotide*** derived from a fungal source, comprising a nucleotide ***sequence*** encoding an enzyme having ***endoglucanase*** activity; (2) An isolated ***polynucleotide*** (II) chosen from a nucleic acid ***sequence*** which encodes or is complementary to a ***sequence*** which encodes (I), a Trichoderma reesei egl6 nucleic acid coding ***sequence*** (S2) or its complement, and a nucleic acid ***sequence*** that hybridizes under stringent conditions to (S2) or its complement or fragment, where (II) encodes a polypeptide having the biological activity of an ***endoglucanase*** ; (3) An ***expression*** construct (III) including a ***polynucleotide*** ***sequence*** having at least 85% ***sequence*** identity to T.reesei egl6 cDNA ***sequence*** (S3), or being capable of hybridizing to a probe derived from (S3) under conditions of intermediate to high stringency, or being complementary to a nucleotide ***sequence*** having at least 85% ***sequence*** identity to (S3); (4) A vector (IV) including (III); (5) A vector (V) comprising (II), operably linked to control sequences recognized by a host cell transformed with the vector; (6) A host cell (VI) transformed with (IV) or (V); (7) A ***recombinant*** host cell (VII) comprising (II); (8) Producing (I); (9) A purified ***recombinant*** enzyme having ***endoglucanase*** activity; (10) a ***recombinant*** host cell comprising a ***deletion*** or insertion or other alteration in the egl6 ***gene*** which inactivates the ***gene*** and prevents EGVI polypeptide production; (11) An antisense oligonucleotide complementary to a messenger RNA that encodes a EGVI polypeptide having (S1), where upon exposure to a ***endoglucanase*** -producing host cell, the oligonucleotide decreases or inhibits the production of ***endoglucanase*** by the host cell; (12) A ***detergent*** composition (VIII) comprising (I); and (13) ***Expressing*** a heterologous polypeptide having beta-glucosidase activity in an Aspergillus sp., involves providing a host Aspergillus with an ***expression*** vector comprising a ***polynucleotide*** encoding a signal ***sequence*** linked to a ***polynucleotide*** encoding a heterologous beta-glucosidase, thus encoding a chimeric polypeptide, and cultivating the host Aspergillus under conditions suitable for the Aspergillus to produce the chimeric polypeptide, where the chimeric polypeptide is produced.

BIOTECHNOLOGY - Preparation: Producing (I), involves stably transforming a host cell with an ***expression*** vector comprising (II), cultivating the transformed host cell under condition suitable for the host cell to produce the ***endoglucanase*** , and recovering the ***endoglucanase*** . The host cell is filamentous fungi or yeast cell (claimed). Preferred ***Polynucleotide*** : In (II), the percentage identity is calculated using the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix. The ***polynucleotide*** is an RNA molecule. (II) encodes an enzyme having ***endoglucanase*** activity, where the enzyme is derived from a Trichoderma source (e.g., T.reesei). Preferred Host Cell: (VI) and (VII) are prokaryotic or eukaryotic cell.

USE - Polypeptide (I) is useful for producing ethanol, which involves contacting a biomass composition with an enzymatic composition comprising (I) to yield a sugar solution, adding to the sugar solution a fermentative ***microorganism*** , and culturing the fermentative ***microorganism*** under conditions sufficient to produce ethanol, where the biomass composition may be optionally pretreated. The method further involves the addition of at least one ***endoglucanase*** or cellbiohydrolase. The pretreatment is with a dilute acid. (I) is also useful for producing ethanol, which involves contacting a biomass composition with an enzymatic composition comprising (I) and a fermentative ***microorganism*** , and culturing the fermentative ***microorganism*** under conditions sufficient to produce ethanol, where the biomass composition may be optionally pretreated (claimed). ***Detergent*** composition (VIII) is useful as softening agent, and also useful for improving the feel of cotton fabrics and for degrading wood pulp into sugars.

EXAMPLE - No relevant example is given. (26 pages)

L10 ANSWER 30 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-17464 BIOTECHDS
TITLE: Cellulose-binding domain-lacking Zygomycetes-originated endoglucanase, with effect of endoglucanase activity enhanced in processing fibers, deinking waste paper and improving freeness of paper pulp;
involving vector plasmid p18-1-mediated gene transfer and expression in Humicola insolens
AUTHOR: NAKANE A; BABA Y; KOGA J; KUBOTA H
PATENT ASSIGNEE: MEIJI SEIKA KAISHA LTD
PATENT INFO: WO 2002042474 30 May 2002
APPLICATION INFO: WO 2000-JP10188 21 Nov 2000
PRIORITY INFO: JP 2000-354296 21 Nov 2000
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
OTHER SOURCE: WPI: 2002-471729 [50]
AN 2002-17464 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A Zygomycetes-originated ***endoglucanase*** lacking the cellulose binding domain, and exhibits ***endoglucanase*** activity, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a protein which is an ***endoglucanase*** originating from a Zygomycetes belonging to family 45 and showing ***endoglucanase*** activity; (2) a protein containing an amino acid ***sequence*** lacking the cellulose-binding domain in a fully defined amino acid ***sequence*** as given in the specification, its ***variant*** , or their analog and exhibiting ***endoglucanase*** activity; (3) a ***gene*** encoding the protein, its ***variant*** , or their analog; (4) an ***expression*** vector containing the ***gene*** ; (5) a host cell transformed with the ***expression*** vector; (6) producing the protein, its ***variant*** , or their analog by culturing the host cells before isolating the product from the cells or cultured material; (7) a protein produced by the method; (8) ***cellulase*** preparations containing the protein, its ***variant*** , or their analog; (9) treating cellulose-containing fibers by contacting such fibers with the protein, its ***variant*** , their analog or the ***cellulase*** preparation; (10) reducing fluffing of cellulose-containing fibers or the start rate of fluffing by contacting with the protein, its ***variant*** , their analog or the ***cellulase*** preparation; (11) improving texture, or appearance, of cellulose-containing fibers with the aim of reducing amount of reprocessing, or for locally changing fiber colors, by contacting such fibers with the protein, its ***variant*** , their analog or the ***cellulase*** preparation; (12) lightening fiber colors by contacting the colors with the protein, its ***variant*** , their analog or the ***cellulase*** preparation; (13) softening cellulose-containing fibers, or for reducing the start rate of hardening, by contacting such fibers with the protein, its ***variant*** , their analog or the ***cellulase*** preparation; (14) ***detergent*** additives containing the protein, its ***variant*** , their analog or the ***cellulase*** preparation in non-flying granule or stabilized liquid form; (15) ***detergent*** compositions containing the protein, its ***variant*** , their analog or the ***cellulase*** preparation; (16) deinking waste paper by using the protein, its ***variant*** , their analog or the ***cellulase*** preparation; (17) improving freeness of paper pulp by treating the pulp with the protein, its ***variant*** , their analog or the ***cellulase*** preparation; and (18) improving digestibility of animal feed by treating the animal feeds the protein, its ***variant*** , their analog or the ***cellulase*** preparation.
BIOTECHNOLOGY - Preferred Host Cell: The host cell is preferably the cell of a mycelioid, e.g. a cell of a ***microorganism*** belonging to the genus Humicoa.
USE - The enzyme can be used in processing fibers, deinking waste paper and improving freeness of paper pulp, which is particularly applicable in ***detergent*** compositions (claimed).
ADVANTAGE - The protein has enhanced ***endoglucanase***

activity.
EXAMPLE - A ***gene*** ***expressing*** a cellulose-binding domain- ***deleted*** RCEI ***mutant*** H43 was constructed by using a codon-optimized ***endoglucanase*** ***gene*** RCEI-containing plasmid p18-1 (WO200024819), which was transferred to Humicola insolens. The required enzyme was ***expressed*** by the transformant for use in treating fibers for evaluation of the ***endoglucanase*** activity. (109 pages)

L10 ANSWER 32 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-11535 BIOTECHDS
TITLE: Novel endoglucanase III (EGIII)-like cellulase variant comprising substitution/deletion at positions corresponding to specific residues in EGIII from Trichoderma reesei, useful for treating cellulose containing textile;
vector-mediated gene transfer and expression in host cell, surfactant, cellulose, site-directed mutagenesis, polymerase chain reaction and DNA primer for use in textile industry
AUTHOR: MITCHINSON C; ROPP T H; SWANSON B A
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2002012464 14 Feb 2002
APPLICATION INFO: WO 2000-US23989 4 Aug 2000
PRIORITY INFO: US 2000-632426 4 Aug 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-241750 [29]
AN 2002-11535 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - An ***endoglucanase*** III (EGIII)-like ***cellulase*** ***variant*** (I) which comprises a ***substitution*** or ***deletion*** at a position corresponding to one or more of residues M79, M154 and/or M118 in EGIII from Trichoderma reesei, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a DNA (II) encoding (I); (2) a vector (III) comprising (II); (3) a host cell (IV) transformed with (III); (4) preparation of (I); and (5) a ***detergent*** composition comprising a surfactant and a ***cellulase*** , where the ***cellulase*** comprises (I) comprising a ***substitution*** at an oxidatively sensitive residue.
BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) in a suitable culture medium under suitable conditions to produce ***cellulase*** which is then obtained (claimed). Preferred ***Variant*** : (I) comprises an amino acid such as leucine, isoleucine, valine, threonine, serine or alanine ***substitution*** at a position corresponding to one or more of residues M79, M154 and/or M118 in EGIII. The ***cellulase*** is derived from a fungus, bacteria or Actinomycete, preferably a filamentous fungus belonging to Euascomycete such as Aspergillus spp., Gliocladium spp., Fusarium spp., Acremonium spp., Myceliophtora spp., Verticillium spp., Myrothecium spp. or Penicillium spp.. Preferably, (I) is an ***endoglucanase*** . Preferred ***Detergent*** : The ***detergent*** is a ***laundry*** ***detergent*** .
USE - (I) is useful in the treatment of a cellulose containing textile (claimed).
ADVANTAGE - By ***substituting*** other amino acids for the native methionines at positions 79, 118 and 154 (sites where oxidation of the enzyme takes place) in EGIII from T. reesei, oxidatively more stable enzymes are obtained.
EXAMPLE - Genomic DNA was prepared for several different ***microorganisms*** to determine whether ***endoglucanase*** III (EGIII)-like ***cellulases*** were encoded by the DNA of a particular organism. Genomic DNA was obtained from Acremonium brachypenium deposit no. CBS866.73, Chaetomium brasillience deposit no. CBS140.50, C. vitellium deposit no.CBS250.85, Emericella desertoru deposit no.CBS653.73, Fusarium equiseti deposit no.CBS185.34, Gliocladium roseum deposit no.CBS443.65, Humicola grisea var. thermoidia deposit no.CBS225.63, Myceliopthora thermophila deposit no. ATCC 48102-48104, Penicillium notatum deposit no.ATCC 9178, 9179, and Phanerochaete chrysosporium deposit no. ATCC28326 and isolated. Site-directed ***mutagenesis*** was performed to incorporate amino acid

substitutions in Trichoderma reesei EGIII. DNA that encoded T. reesei EGIII was amplified from a cDNA clone using PCR primers that introduced a BglII restriction endonuclease site at the 5' end of the egl3 ***gene*** (immediately upstream of the first ATG codon) and an XbaI site at the 3' end (immediately downstream of the stop codon). The amplified fragment was then digested with BglII and XbaI, and ligated into pUC19 digested with BglII and XbaI. ***Variants*** were made in this plasmid using the QuikChange ***mutagenesis*** methods. The ***variant*** genes were then subcloned into the Aspergillus ***expression*** vector pPGPT-pyrG. Vectors carrying the ***variant*** genes were then transformed into A. niger var. awamori and the resultant strains grown in shake-flask cultures. EGIII ***variants*** (M79A, M79C, M79I, M118A, M118T, M118L, M118I, M118N, M118S, M118V, M118D, M118G, M118C, M118H, M118E, M154A, M154T, M154N, M154C, M154Q) were then purified from cell-free supernatants of these cultures by column chromatography. The EGIII-like ***cellulase*** containing supernatants were treated overnight with 0.18 mg/ml of ***endoglucanase*** H at 37 degrees Centigrade. Ammonium sulfate was added to the treated supernatants to a final concentration of 0.5 M. After centrifugation, the supernatant was loaded onto the column. The column was then ***washed*** with 3 volumes equilibration buffer and then eluted. Each volume of flow through was collected as a separate fraction with the EGIII-like ***cellulase*** appearing in the second fraction. To assay for specific activity of EGIII-like ***cellulases*** , a nitrophenyl O-D-cellobioside (NPC) hydrolysis assay was used. In a microtiter plate, 100 microlitres 50 mM sodium acetate, pH 5.5 and 20 microlitres of 25 mg/ml o-NOC (o-nirophenyl o-D cellobioside) in assay buffer was added. The plate was incubated for 10 minutes at 40 degrees Centigrade. Once equilibrated, 10 microlitres EGIII-like ***cellulase*** was added and the plate incubated. To quench the hydrolysis and stop the reaction, 70 0.2 M glycine was added. The plate was then read in a microtiter plate reader at 410 nm. The concentration of EGIII-like ***cellulase*** was determined by absorbance at 280 nm. The melting point of the EGIII ***variants*** was determined by equilibrium CD. Spectra were collected from 265-210 nm. Thermal denaturations were performed at 217 nm from 30-90 degrees Centigrade with data collected every 2 degrees. The equilibration time at each temperature was 0.1 minutes and data was collected for 4 seconds/sample. The remainder of the pH 8 sample was divided into 5 x 400 microlitres aliquots. Two samples were adjusted to pH 5 and 7 with acetic acid and two others were adjusted to pH 9 and 10 with sodium hydroxide. Thermal denaturations of all 5 samples were performed simultaneously. The melting points were determined. The ***substitutions*** with other amino acids significantly decreased specific activity of the EGIII ***variants*** .(41 pages)

L10 ANSWER 35 OF 41 USPATFULL on STN
ACCESSION NUMBER: 2001:93472 USPATFULL
TITLE: Enzyme granulate for washing and cleaning
INVENTOR(S): Herrmann, Hubert A., Cremlingen-Weddel, Germany, Federal Republic of
Spannagl, Rolf, Husum, Germany, Federal Republic of
PATENT ASSIGNEE(S): Genencor International, Inc., Palo Alto, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6248706	B1	20010619
	WO 9743482		19971120
APPLICATION INFO.:	US 1999-180181		19990331 (9)
	WO 1997-US7982		19970513
			19990331 PCT 371 date
			19990331 PCT 102(e) date

	NUMBER	DATE
PRIORITY INFORMATION:	DE 1996-19619221	19960513

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Krynski, William

ASSISTANT EXAMINER: Garrett, Dawn L.
LEGAL REPRESENTATIVE: Genencor International, Inc.
NUMBER OF CLAIMS: 38
EXEMPLARY CLAIM: 1
LINE COUNT: 1159
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The preparation of an activity-stable and low-dust enzyme granulate for washing and cleaning applications, e.g. for use in granular washing and cleaning agent compositions, is described. Also described are the activity-stable and low-dust enzyme granulates obtained in accordance with the method of preparation as well as their use in washing and cleaning applications. In addition, in a special aspect of the invention, the use of specially selected flours as auxiliary agents for the preparation of enzyme granulates for diverse application purposes is described.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L10 ANSWER 39 OF 41 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1996-02391 BIOTECHDS
TITLE: Detergent composition for use for washing wool and silk fabric;
surfactant composition containing a polymeric dye transfer-inhibitor and a mutant alkaline protease, especially Bacillus lentus mutant subtilisin, prepared by enzyme engineering
AUTHOR: Baeck A C; Busch A; Verschuere A K M
PATENT ASSIGNEE: Procter+Gamble
LOCATION: Cincinnati, OH, USA.
PATENT INFO: EP 687733 20 Dec 1995
APPLICATION INFO: EP 1994-870096 16 Jun 1994
PRIORITY INFO: EP 1994-870096 16 Jun 1994
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1996-031779 [04]
AN 1996-02391 BIOTECHDS
AB A new surfactant composition substantially free of bleach comprises a polymeric dye transfer-inhibitor (polyamine N-oxide polymer, copolymer of N-vinylpyrrolidone and N-vinylimidazole, polyvinylpyrrolidone, polyvinyloxazolidone, polyvinylimidazole or their mixtures) and a ***mutant*** of a highly ***alkaline*** protease (I) with at least 70% homology with the disclosed protein ***sequence*** and differing by at least 1 ***mutation*** at nucleotides 96-110 and 123-135. (I) has 1 ***mutation*** of valine-104 to isoleucine or tyrosine and 1 ***mutation*** at position 123 of asparagine to serine and may have other ***mutations*** within and/or outside these regions. (I) preferably has a combination of ***mutations*** at positions 27, 76, 99, 101, 103, 222 and/or 274 e.g. K27R, N76D, S99D, S101R, S103A, M222S, M222A, M222C and/or T274A. The composition may also include a ***cellulase*** (EC-3.2.1.4) and/or a peroxidase (EC-1.11.1.7). Also new is a method for preparing a ***mutant*** proteins which involves culturing a ***microorganism*** host strain transformed with an ***expression*** vector containing DNA encoding the ***mutant*** (I) and recovering the ***mutant*** (I). (I) is preferably Bacillus lentus subtilisin (EC-3.421.62). (23pp)

L10 ANSWER 41 OF 41 USPATFULL on STN
ACCESSION NUMBER: 90:15487 USPATFULL
TITLE: DNA fragments containing alkaline cellulase gene, recombinant plasmids with said DNA fragments inserted therein, and recombinant microorganisms
INVENTOR(S): Ozaki, Katsuya, Utsunomiya, Japan
Oshino, Kazushi, Utsunomiya, Japan
Koike, Kenzo, Utsunomiya, Japan
Ito, Susumu, Utsunomiya, Japan
Okamoto, Kikuhiko, Koshigaya, Japan
PATENT ASSIGNEE(S): KAO Corporation, Tokyo, Japan (non-U.S. corporation)

NUMBER	KIND	DATE

PATENT INFORMATION: US 4904599 19900227
APPLICATION INFO.: US 1987-109510 19871019 (7)

	NUMBER	DATE
PRIORITY INFORMATION:	JP 1986-259923	19861031

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Teskin, Robin
ASSISTANT EXAMINER: Burrous, Beth
LEGAL REPRESENTATIVE: Oblon, Spivak, McClelland, Maier & Neustadt
NUMBER OF CLAIMS: 9
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 13 Drawing Figure(s); 10 Drawing Page(s)
LINE COUNT: 678
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB DNA frangments containing an alkaline cellulase K gene, which comprise about 4.0, about 2.4 and about 1.9 kilo base pairs, respectively, each having a specific restruction map, recombinant plasmids containing any of these DNA fragments, and recombinant microorganisms harboring any of said plasmids.

The alkaline cellulase K gene is derived from a bacterial strain which belongs to the genus Bacillus and is alkalophilic, that is, capable of revealing an optimum growth in an alkaline pH region.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d his

L1 QUE ((ENDOGLUCAN(S) HYDROLASE#) OR (CARBOXYMETHYL(S) CELLULASE#

L2 110974 S L1
L3 17599 S (GENE OR SEQUENCE OR POLYNUCLEOTIDE OR RECOMBINANT)(S)L2
L4 4326 S (MUTA? OR VARIA? OR MODIFI? OR SUBSTITUT? OR DELET?)(S)L3
L5 0 S EXPREES? (S)L4
L6 1632 S EXPRESS? (S)L4
L7 216 S (WASH? OR DETERGENT? OR ALKAL? OR LAUNDR?)(S)L6
L8 45 S MICROORGANISM# (S) L7
L9 0 S HEMICOLA (S) L8
L10 41 DUP REM L8 (4 DUPLICATES REMOVED)

=> log y